2001

Laboratory denitrification using sediments from the Elk Valley Aquifer

Alison E. Kammer
University of North Dakota

LABORATORY DENITRIFICATION USING SEDIMENTS FROM THE ELK VALLEY AQUIFER

by

Alison E. Kammer

Bachelor of Science, University of Wisconsin - River Falls, 1997

A Thesis

Submitted to the Graduate Faculty

of the

University of North Dakota

in partial fulfillment of the requirements

for the degree of

Master of Science

Grand Forks, North Dakota

May

2001

This thesis, submitted by Alison Elizabeth Kammer in partial fulfillment of the requirements for the Degree Master of Science from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

Scott F. Korom

(Chairperson)

Richard D. LeFever

Frank R. Karner

This thesis meets the standards for appearance, conforms to the style and format requirements of the Graduate School of the University of North Dakota, and is hereby approved.

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Department	Geology and Geological Engineering
Degree	Master of Science

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ACKNOWLEDGMENTS

I want to extend my most sincere appreciation to my committee chairperson, Dr. Scott F. Korom, for his commitment to education, enthusiasm and energy for research, and unwavering direction and encouragement throughout this project. To my committee, Dr. Richard D. LeFever and Dr. Frank R. Karner, I thank you for the encouragement, advice, and assistance throughout the course of my graduate studies.

Financial assistance for this project was provided by a USEPA Section 319 grant with matching funds by the North Dakota Department of Health, North Dakota State Water Commission, and the University of North Dakota. I would like to thank Dr. William Gosnold, who encouraged me to attend the University of North Dakota and provided funding for my first three semesters.

Others who have lent their skills, advice, and encouragement include Dean Grier, the manager of the Materials Characterization Laboratory at North Dakota State University, David Hassett of the Energy and Environmental Research Center, and last, but not least, Allen Schlag, manager of the Water Quality Laboratory at the University of North Dakota, who conducted the background research for this project and provided hours of technical assistance in the lab. His commitment to excellence and friendship are greatly appreciated by myself and everyone who works with him.

Thank you to my family for the guidance and support that have helped me become the person I am. I couldn't have done it without you.

ABSTRACT

An experiment was conducted to determine if a decrease in the content of sulfides related to denitrification could be measured in the laboratory using sediments of the Elk Valley Aquifer in eastern North Dakota. The experiment entailed analyzing sediments for sulfides and solid organic carbon and isolating them in sample containers with a nitrate solution to determine if a measurable decrease in these components could be observed.

Previous denitrification studies in the Elk Valley Aquifer showed an increase in sulfate in conjunction with a decrease in the concentration of nitrate. Sulfate is a by-product of autotrophic denitrification where sulfide is used as the electron donor by anaerobic bacteria to convert nitrate to harmless nitrogen gas.

Results of the laboratory study show that denitrification did occur in the samples, evidenced by the decrease in nitrate concentration and an increase in the concentration of dissolved inorganic carbon. Dissolved inorganic carbon is a by-product of heterotrophic denitrification where anaerobic bacteria utilize solid organic carbon as an electron donor. This finding is significant because it shows that sediments studied in-situ in the Elk Valley Aquifer reacted differently when they were studied in the laboratory.

Calculations were made to show the total amount of denitrification that would be possible based strictly on the content of sulfides and solid organic carbon. Conclusions based on rates of denitrification previously measured in the Elk Valley Aquifer and the theoretical potential of denitrification based on the electron donor content found in this study, suggest that the availability of electron donors in the sediments may be limited in regard to bacterially mediated denitrification.

INTRODUCTION

Nitrate is a common groundwater contaminant (Freeze and Cherry, 1979) that can affect shallow unconfined aquifers such as the Elk Valley Aquifer (EVA) in eastern North Dakota. Nitrate contaminated groundwater is a public health concern because it is a suspected carcinogen and can cause a potentially lethal condition in infants called methemoglobinemia (Canter, 1997). The US drinking water standard for nitrate in drinking water, reported as nitrate-nitrogen (NO_3^--N), is 10 mg/L. Sources of nitrate in groundwater include nitrogen fertilizers, livestock waste, and septic systems (Korom, 1992).

Groundwater nitrate concentrations may be attenuated by denitrification where nitrate, serving as an electron acceptor, is reduced to harmless nitrogen gas. Such reactions require electron donors. Identifying the electron donors contributing to denitrification and quantifying their availability are essential in assessing the denitrification capacity of an aquifer (Korom, 1992).

Schlag (1999) used an in-situ tracer test to identify the major electron donors contributing to denitrification in the EVA. His research showed that the loss of nitrate in groundwater was accompanied by an increase in sulfate concentration. He concluded that sulfide was an important electron donor in the EVA. In his study, correlating the

sulfate increases in groundwater to a loss of sulfides in the sediments was impractical; therefore, this follow-up study was initiated. Using sediment samples from Schlag's field site, a laboratory batch experiment was designed to replicate the field results while permitting the sediments to be monitored. The hypothesis was that denitrification could be correlated with a measurable decrease in sediment sulfide concentrations.

BACKGROUND

Denitrification

There are four requirements for denitrification. They are 1) suitable electron donors, 2) anaerobic conditions, 3) the presence of bacteria possessing the metabolic capacity, and 4) the presence of N oxides as terminal electron acceptors (Firestone, 1982). How much nitrate an aquifer is capable of converting to nitrogen gas is thought to be dependent on the quantity of available electron donors (Korom, 1992).

One suitable electron donor for denitrification is sulfide. Sulfide compounds are an example of inorganic electron donors used in denitrification and are grouped into two categories. The first group is monosulfides, also known as acid volatile sulfides. Minerals in the monosulfide group include griegite (magnetic Fe_3S_4), mackinawite ($Fe_{1.11}S$), galena (PbS), sphalerite (ZnS), and pyrrhotite (magnetic $Fe_{0.9}S$). The term acid volatile sulfide is in reference to the solubility of these minerals in a non-oxidizing acid, such as HCl (Canfield et al. 1986, Rice et al. 1993). The second group is disulfides. The disulfide group consists of the minerals such as marcasite (orthorhombic FeS_2) and pyrite (cubic FeS_2). The latter has been identified in the EVA using X-ray diffraction. The formation of pyrite is widespread and common, especially in anaerobic conditions that contain organic material. Pyrite is the most common disulfide mineral (Berner, 1970).

Although cations such as lead and zinc can be associated with sulfides the iron sulfide minerals are the most common because the conditions in which they are formed are also common. (Berner, 1970).

The sedimentary environment that allows for the formation of pyrite also contains another common electron donor: organic carbon. It is commonly assumed that the electron donor responsible for denitrification in most aquifers is organic carbon. The organic carbon utilized in denitrification must be labile and can either be dissolved in the groundwater or present in solid form. Both organic carbon and sulfide are commonly found in anaerobic conditions, a requirement for denitrification.

An example of denitrification with pyrite as an electron donor is given by Kölle et al. (1985) as

$$5\ FeS_2 + 14\ NO_3^- + 4\ H^+ \rightarrow 7\ N_2 + 10\ SO_4^{-2} + 5\ Fe^{2+} + 2\ H_2O \qquad (1)$$

The reaction shows the sulfide atoms in pyrite as the electron donor and nitrate as the electron acceptor. Sulfate (SO_4^{-2}) and ferrous iron (Fe^{2+}) are products. The ferrous iron that has been produced in this reaction is also an electron donor and can also be utilized for denitrification as follows

$$5\ Fe^{2+} + NO_3^- + 7\ H_2O \rightarrow 5\ FeOOH + 0.5\ N_2 + 9\ H^+ \qquad (2)$$

This reaction results in the formation of goethite (FeOOH). Combining Reactions (1) and (2) gives

$$2\ FeS_2 + 6\ NO_3^- + 2\ H_2O \rightarrow 3\ N_2 + 2\ FeOOH + 4\ SO_4^{-2} + 2\ H^+ \qquad (3)$$

Reaction (3) shows the complete reaction between pyrite and nitrate in denitrification and is the equation used to determine the theoretical maximum autotrophic denitrification potential due to pyrite for the EVA.

An example of denitrification with organic carbon (glucose) as an electron donor is

$$4\ NO_3^- + 5/6\ C_6H_{12}O_6 \rightarrow 2\ N_2 + H^+ + 5\ HCO_3^- + 2\ H_2O \qquad (4)$$

Each of the four chemical reactions is carried out through a series of bacterially mediated processes. Bacteria capable of metabolizing nitrate can either be autotrophs or heterotrophs. Autotrophs do not require organic carbon for growth and maintenance and can obtain the carbon necessary for life from inorganic carbon dioxide (CO_2). Autotrophs possess the ability to use inorganic electron donors, such as sulfides, for metabolism (Chapelle, 2001). Examples of autotrophic denitrification are given in Reactions (1 - 3). Heterotrophs use organic carbon for metabolism and as a carbon source for growth and maintenance. Reaction (4) is an example of heterotrophic denitrification. The bacteria that have the capacity to denitrify are obligate anaerobes or facultative anaerobes. Obligate anaerobes can only survive in the absence of oxygen. Facultative anaerobes use oxygen when it is available, but are also capable of using other oxidants, such as nitrate, when oxygen is limited (Chapelle, 2001).

The requirements and occurrence of denitrification have been studied in the field and in the laboratory. Studies include observations of the products and reactants given in

Reactions (1) through (4), sediment/water interactions, and in-situ tracer tests.

Sediment Characteristic of Denitrifying Aquifers

Postma et al. (1991) investigated denitrification in an aquifer below areas with different land use. Land use is a factor in the amount of nitrate reaching the groundwater. The aquifer is located in western Denmark and consists of Pleistocene sand deposits from a braided river system. Miocene deposits that contain brown coal fragments underlie this sand and have been reworked into the Pleistocene sand deposits. The aquifer material is predominantly medium to coarse-grained quartz sand. Chemical weathering has removed $CaCO_3$ from at least the upper 30 meters of the aquifer. Sediment was analyzed for total carbon, total organic carbon, and total inorganic reduced sulfur. Reduced sulfur species were analyzed using a chromium reduction method. Results were reported as pyrite and the average content was 3.6 mmol/kg (0.41 % by weight). Organic carbon concentrations are shown graphically to be greater than pyrite. It is believed that pyrite is the main electron donor in regard to denitrification, because the organic carbon is not energetically favorable to the bacterial population for utilization.

Kölle et al. (1985) did an experiment to determine the maximum reactive portion of pyrite in the Fuhrberg Feld Aquifer sediments in Hannover, Germany. The aquifer is unconfined and underlain by low permeability clay. Sediments in this aquifer consist of carbonate-free sand and gravel. Sediment samples contain lignite grains and microcrystalline pyrite. Pyrite can be found associated with the lignite and as individual

black stains, particles, coatings, and crusts on quartz grains. The reactive portion of pyrite was studied by placing sediment in a nitrate solution and measuring the increase in sulfate concentration. This was termed the Sulfate Formation Capacity (SFC). Analyses showed that 2 – 20 kg of sulfate could be generated per cubic meter of sediment material. Calculations show that recharge equal to 200 mm per year, with a nitrate (NO_3^-) concentration of 100 mg/L, can be denitrified by pyrite oxidation over a period of about 1000 years. No data were given for observed decreases in nitrate concentrations during the SFC test, therefore the increase in sulfate cannot be directly correlated to decreases in nitrate. The SFC test did not determine if the increase in sulfate was due to the presence of nitrate in solution or due to the presence of other oxidants. The SFC test did show that not all of the sulfide present in the sediments was oxidized while exposed to the solution, suggesting not all of sulfide present is easily oxidized.

Aravena and Robertson (1998) evaluated denitrification in groundwater on the north shore of Lake Erie using an isotope tracer test. The aquifer has a saturated thickness of 5 m and overlies a flat lying silt unit. The sediment consists mainly of quartz and carbonate minerals. The average organic carbon content was 0.15% by weight and the sulfur content was 0.02% by weight. Aravena and Robertson (1998) also used the isotope ratio of $^{34}S/^{32}S$ to determine that biogenic pyrite is an electron donor at this site. This study concluded that ~25% of the denitrification was autotrophic and ~75% was heterotrophic.

Previous Studies in the EVA

The ability of the EVA to naturally attenuate nitrate has been observed by Mayer (1992), Patch and Padmanaben (1996), and Schlag (1999). Mayer (1992) observed decreasing nitrate concentrations with depth in the EVA. He concluded the active zone of heterotrophic denitrification was 1.5 - 3 m below the water table. Evidence for heterotrophic denitrification at this depth were reduced nitrate concentrations, low dissolved oxygen concentration (less than 1.0 mg/L), and a detectable biological oxygen demand, which indicates that there is metabolically available organic carbon for heterotrophic bacteria. Mayer proposed that autotrophic denitrification could be responsible for the decrease in nitrate below the water table because the source of metabolically available dissolved organic carbon became depleted. Although the occurrence of autotrophic denitrification was not established, it was suggested that its potential to occur was significant and could be used to explain the increasing sulfate concentrations in the groundwater.

Patch and Padmanabhen (1996) conducted a study to determine the cause of the vertical nitrate gradient present in the EVA. Observations of this study included a decrease in nitrate concentration and increase in the isotopic enrichment of $^{15}N/^{14}N$ with depth. Heterotrophic denitrification, not dilution or dispersion, was the mechanism used to explain these observations.

Schlag performed an in-situ tracer test using specially designed chambers called in-situ mesocosms (ISMs). Observations from this experiment showed that 61% of the

nitrate attenuated within the ISMs could be accounted for by autotrophic denitrification. Evidence for denitrification included the isotopic enrichment of $^{15}N/^{14}N$, a concomitant increase in sulfate concentrations with the loss of nitrate , as shown in Reaction (1), and a decrease in dissolved iron , as shown in Reaction (2). These tracer tests were done in the same location that the sediments were collected for this laboratory denitrification study.

Previous to this study, others only speculated on the quantity of electron donors present in the EVA. The speculations warranted further research in identifying and quantifying suspected types of electron donors, understanding factors in their utilization, and determining optimum conditions for denitrification to occur. Up to this point, there has been no correlation between a documented in-situ and laboratory investigation of denitrification using aquifer materials from the same location.

METHODS

Sample Collection and Experiment Design

An experiment was designed to determine the quantity of sulfides being used in denitrification in a laboratory setting. An auger (Gidding's Probe) was used to collect sediments from the Elk Valley Aquifer at three depth intervals at each of three locations near the ISMs installed by Schlag (1999). The study site is approximately 1 km west of Larimore, North Dakota on the west side of T. 151 N., R. 55 W., SW 1/4 SE 1/4 Section 1.

The depth intervals were 2.3-3.1 m, 4.9-5.8 m, and 6.7-7.9 m below ground surface. The depths were chosen for their proximity to the water table , the depth of the ISMs used by Schlag (1999), and a depth thought to have minimal exposure to nitrate and a higher content of inorganic electron donors, respectively. After collection, the sediments were kept wet and handled in a nitrogen atmosphere to avoid oxygen contamination, which could oxidize the sulfides or kill obligate anaerobic denitrifying bacteria present in the sediments (Chapelle, 2001). The sediments were ground in a corundum mortar and pestle to reduce particle size and increase the surface area of the electron donating species. Next, the sediments were placed in sample containers with a nitrogen headspace. Representative samples were dried and analyzed for solid organic

carbon (SOC), inorganic sulfides (S^-), and general mineral identification using X-ray diffraction (XRD).

The experiment began on February 1, 2000 with the addition of water to the sample containers and ended 210 days later, on August 29, 2000. Samples from each depth were incubated in six sets of containers with a nitrate solution. The nitrate solution was made using water collected from a monitoring well screened at 6.0-7.5 m below ground surface (David Rush, Energy and Environmental Research Center, personal communication). This monitoring well is located directly north of the sediment sampling and ISM site. The water collected from the monitoring well was spiked with potassium nitrate (KNO_3) and potassium bromide (KBr). Bromide was used as a tracer to determine if evaporation or anion adsorption was occurring in the sample containers. Bromide was chosen because its concentration in the water is naturally low and because it has similar charge characteristics as nitrate (Faure, 1998).

Each of the six sets had three replicates from each of the three depths, for a total of 54 sample containers. Having three replicates permits the determination of average values and minimizes the effects of sediment heterogeneity. Four additional containers were used as controls. Three controls consisted of sediment from each depth and native water (Ctrl 2.3-3.2, Ctrl 4.9-5.8, and Ctrl 6.7-7.9). Native water, defined as water taken from the monitoring well and not altered chemically in the lab, was added to the control samples. The fourth control container had amended water with no sediments (Ctrl H2O).

Controls were used to determine changes in water chemistry that are not a result of denitrification.

One of the six set of containers was analyzed every 30-45 days. At the same collection intervals, water samples were taken from the controls. Analyses were done to determine the amount of nitrate-nitrogen (NO_3^--N), sulfate (SO_4^{-2}), bromide (Br^-), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), pH, and dissolved oxygen (DO) present in the water. The sediments in each sample container were tested for sulfide (S^-) and solid organic carbon (SOC) content. In the controls, S^- and SOC were only done on day 1 and days 210 because these were the only times sediments were taken from these containers.

On day 93 of the study, 0.5 mL of sodium phosphate monobasic ($NaH_2PO_4.H_2O$) with a concentration of 1000 mg/L PO_4^{-3}-P was injected into each sample container to provide nutrients in an attempt to stimulate denitrification. The ending concentration in each sample container was ~1 mg/L PO_4^{-3}-P.

Sampling Protocol

One set of samples plus the four controls were opened and tested for DO and pH every 30-45 days. At this time, 30-60 mL of solution were collected from each container with a syringe, filtered with a 0.45-μm pore space syringe filter and placed immediately in a 10 mL Shimadzu™ sampling tube. The tubes were capped with no head space for DIC and DOC analysis. The rest of each sample was filtered, placed in a sample

container, and refrigerated overnight at 4°C. The samples were analyzed for NO_3^--N, SO_4^{-2}, and Br^- concentrations within 24 hours. All analyses were performed at the University of North Dakota Water Quality Laboratory (WQL).

While aqueous samples were collected from each container, the containers remained in the nitrogen glove bag. They were recapped as soon as possible. Opening the glove bag to collect samples allowed oxygen to enter. The oxygen contamination in the glove bag, along with the headspace created in the control containers due to the removal of solution out of the caused some oxygen contamination to occur. Oxygen contamination was not a problem in the other experimental containers used in the study because they were not opened at every sample collection interval.

The total volume of water in each container was measured before it was discarded. To measure the amount of water trapped in the sediments in each container, the sediments were carefully collected and weighed wet. They were dried in an oven at 90°C. The sediments were weighed again to determine the weight loss due to water evaporation. The weight of the water lost to evaporation was converted to volume using the estimate that 1.0 mL of water weighs 1.0 gram.

Sediment from each container was divided into two sub-samples. One sub-sample was weighed, placed in a 250 mL amber glass bottle, treated with 25 ml of 5% HCl to remove the carbonates in the sediments and stored overnight. The acid was then decanted and deionized water was added to rinse the sediments. Three rinses were done to remove the acid from the sediments. The sediments were dried and weighed again to

determine carbonate content. The treated sub-samples were analyzed for acid non-purgeable SOC, so called because of the pretreatment with HCl. The SOC reading were adjusted to account for the missing weight of the carbonates. The method used for the adjustment is shown in Appendix A. The second sub-sample was placed in a separate container and analyzed for S^- content.

Analytical Methods

Analytical methods used with references are shown in Table 1. To insure the accuracy of the measurements, procedures for sample storage and analysis from Standard Methods for the Examination of Water and Wastewater (APHA, 1998) were followed. When using calibration curves, at least one standard of higher and one of lower concentration than the samples were used. Quality assurance/quality control (QA/QC) procedures included running duplicate samples, analysis of known standards and blanks, as well as running each depth tested in triplicate to obtain averages and standard deviations. QA/QC procedures indicated the data are reliable.

XRD analyses of the sediments were performed at the Material Characterization Laboratory at North Dakota State University. JADE 3.1 (Materials Data, Inc., 1997) was used for mineral identification.

Table 1. Methods used to perform analyses with references.

Analyte	Detection Limit	Method	Equipment	Reference
NO_3^--N, Br^-, SO_4^{2-}	1.0 mg/L* 2.0 mg/L* 1.5 mg/L*	Method 4110 B (modified), Ion chromatography with chemical suppression of eluent conductivity	Alltech™ Ion Chromatograph, Hamilton™ PRP-X100 analytical column, HP™ 3396 integrator, 4.0 mM p-Hydroxybenzoic Acid at 2.0 mL/min	APHA, 1998
Total dissolved carbon, Dissolved inorganic carbon, Dissolved organic carbon	1.0 mg/L* 1.0 mg/L* 1.0 mg/L*	Method 5310 B, High temperature combustion with a non-dispersive infrared gas analyzer	Shimadzu™ TOC 5050 Analyzer	APHA, 1998
Dissolved Oxygen	0.1 mg/L	Method 4500-O G, Membrane Electrode Method	YSI™ Model 57 dissolved oxygen meter	APHA, 1998
pH	n/a	Method 4500 B, Electrometric Method	Orion™ Model 250A portable meter, Orion™ Model 9107 pH Triode electrode	APHA, 1998
Sediment sulfide	0.01% by weight	Chromium Reduction and Method 4500-S2- E (modified), Iodometric Method	Glassware	APHA, 1998; Canfield et al., 1986
Sediment Organic Carbon	<0.01% by weight	High temperature combustion with a non-dispersive infrared gas analyzer	Shimadzu™ TOC 5050 Analyzer, Shimadzu™ SSM 5000	Churcher, and Dickout, 1987
Sediment Inorganic Carbon	n/a	HCl dissolution, measured weight reduction	n/a	Mermut, and Arshad, 1987
Sediment	n/a	X-ray diffraction	Philips™ X'Pert Multi-purpose Diffractometer (MPD), Long line focus Cu X ray tube, and a sealed proportional counter.	Moore, and Reynolds Jr, 1989

* As reported by the University of North Dakota Water Quality Laboratory.

RESULTS AND DISCUSSION

Sediment Characteristics at the EVA Field Site

X-ray diffraction (XRD) was performed on sediment samples from each depth studied. Common minerals and mineral groups identified throughout the aquifer from depths of 2.3-7.9 m include quartz (SiO_2), potassic feldspar (group of minerals, example - microcline $KAlSi_3O_8$), plagioclase feldspar (albite - anorthite solid solution series $NaAlSi_3O_8$ - $CaAl_2Si_2O_8$), calcite ($CaCO_3$), dolomite ($CaMg(CO_3)_2$), smectite (group of minerals, example - montmorillonite $[(Al_{1.77}Fe^{3+}_{0.03}Mg_{0.20})\ (Si_{3.74}Al_{0.26})O_{10}(OH)_2]$), hydrated silica ($SiO_2 \cdot nH_2O$), mica-illite ($[K_{0.6}Mg_{0.25}Al_{2.3}Si_{3.5}O_{10}(OH)_2]$), kaolinite ($Al_2Si_2O_5(OH)_4$), and amphibole (group of minerals, example - hornblende $[(Ca,Na)_{2\text{-}3}(Mg,Fe^{2+},Fe^{3+},Al)_5(Al,Si)_8O_{22}(OH)_2]$). One small peak found at the two lowest depth intervals indicated that pyrite (FeS_2) may be present in small amounts. No other sulfide minerals were indicated using XRD. No sulfate-bearing minerals were identified, which would potentially be a source of dissolved SO_4^{-2} in the water. Faure (1998) and Motanna et al. (1978) were used for the mineral formulas. XRD scans are in Appendix B.

Schlag (1999) recorded grain size analyses from depths of 2.3-6.4 meters at this site. It was determined that a majority of the sediment at the site is fine sand (~68-79%) mixed with silt (~2-15%), and clay (~11-16%).

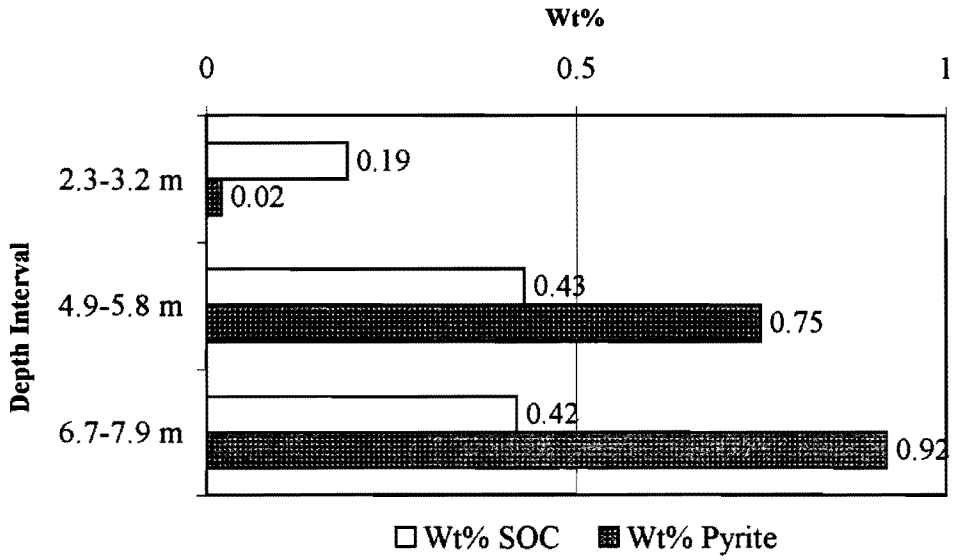
Sediment descriptions were done for each of the depth intervals studied. The sediments at 2.3-3.2 meters are rusty brown, well sorted, fine-grained, mostly sand, mixed with silt and clay, and wet. Some mottling begins to occur at approximately 3 meters. The Munsell color of the sediments when wet is 10YR 4/3 (Munsell Soil Color Charts, 1994). Chemical analyses were performed three times and show and average SOC content of 0.19 ± 0.01 wt% and an average S^- content of 0.01 ± 0.01 wt% (0.02 wt% FeS_2 equivalent).

At 4.9-5.8 meters, the sediments are gray to greenish gray, well sorted, fine-grained, mostly sand mixed with silt and small amounts of clay, and wet. The Munsell color of the wet sediments is 2.5Y 3/1 (Munsell Soil Color Charts, 1994). Chemical analyses were performed three times and show an average SOC content of 0.43 ± 0.10% by weight and an average S^- content of 0.41 ± 0.02% by weight (0.75 wt% FeS_2 equivalent).

The sediments at 6.7-7.9 m are dark gray with some mottling of lighter color, well sorted, medium-grained, mostly sand with some silt and little clay, and wet. Sand grains broke apart into silt and clay sized particles. The Munsell color of the sediments when wet is 5Y 2.5/1 (Munsell Soil Color Charts, 1994). Chemical analyses were performed three times and showed an average SOC content of 0.42 ± 0.02% by weight and an

average S^- content of 0.49 ± 0.01% by weight (0.92 wt% FeS_2 equivalent). The quantity of electron donors in the form of organic carbon and sulfide is greatest at this depth. Figure 1 shows the average content of SOC and FeS_2 measured in the sediments at each depth interval before a nitrate solution was added to each set of samples.

Figure 1. Content of pyrite and SOC at each depth interval.



Aqueous Analysis on Day 1

Water for the experiment was collected from monitoring wells in the EVA north of the sediment collection site at a screened depth of 6.0-7.5 m. Analyses showed the native water had non-detectable amounts of NO_3^--N and Br^- and 56.7 mg/L SO_4^{2-}. The concentration of the DIC was 71.1 mg/L. The amended water was analyzed and had a

concentration of 104 mg/L NO_3^--N, 55.1 mg/L SO_4^{-2}, and 21.9 mg/L Br^-. All analytical data can be found in Table 2 in Appendix C.

It was assumed at the start of the experiment that each sample container from the same depth interval would respond in a similar manner as the other samples in that interval. At the conclusion of the experiment, it was observed that each of the samples responded in a different manner for unknown reasons. Because of this, only the sample from each depth interval that showed the greatest loss of NO_3^--N was used to calculate the ratio of autotrophic and heterotrophic denitrification that occurred.

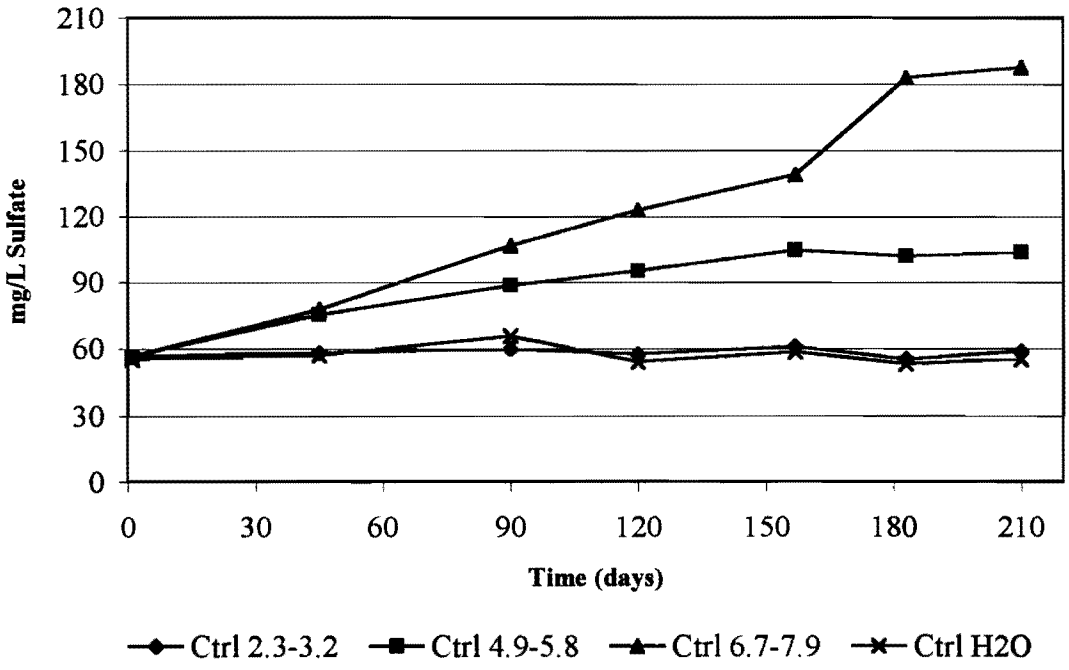
Statistical analysis was done on the slope of the line created by the average values of the analytes to determine if the concentrations had significantly changed over time. Average values with one standard deviation are found in Table 3 in Appendix C. A 90% confidence interval was used to determine if any of the trends seen were statistically significant. Significant increases and decreases are related to the deviation of the slope of the line from zero. A significant increase has a positive deviation from zero, a significant decrease has a negative deviation from zero, and a trend with no significance shows no discernable deviation of the slope from zero (Krumbein and Graybill, 1965).

Control Samples

Using the control samples to determine the change in aqueous chemistry proved useful to the interpretation of the results of this study. Samples Ctrl 2.3-3.2, Ctrl 4.9-5.8, and Ctrl 6.7-7.9 had no detection of NO_3^--N or Br^- throughout the 210-day study. SO_4^{-2}

increased 1.9 mg/L in Ctrl 2.3-3.2., 47.3 mg/L in Ctrl 4.9-5.8, and 131 mg/L in Ctrl 6.7-7.9. Figure 2 shows the change in SO_4^{-2} in the control samples at each depth. The change in the concentration of SO_4^{-2} in the control samples is significant because it shows that the increasing SO_4^{-2} in the experimental samples may not be related to denitrification.

Figure 2. Concentration of sulfate in control samples for each depth interval.



It is likely that the increase in SO_4^{-2} in the control samples in due to oxidation by oxygen. The introduction of oxygen into the control samples was due to the glove bag being ineffective in keeping oxygen out during sample collection. Each time aqueous samples were collected from the control containers, the increasing headspace in the

sample container allowed oxygen to mix with the sample. This problem was not encountered in the experimental samples because the samples were kept sealed until the final analyses were performed on each sample set. Dissolved oxygen data for each sample in found in Appendix C.

Oxidation of sulfides in the control samples may be comparable to the SFC test done by Kölle et al. (1985), except that oxygen was used as the oxidant instead of nitrate. Figure 1 also shows the increase in SO_4^{-2} leveled off after 157 days in Ctrl 4.9-5.8, indicating that sulfide availability may be limited.

The content of sulfide measured in the sediments of the control samples at the beginning and end of the experiment showed a decrease at the two lower depth intervals. This is the only decrease of sulfides observed throughout the experiment; however, because only two data points exist, no inferences about its significance can be made.

Denitrification in the Experimental Samples

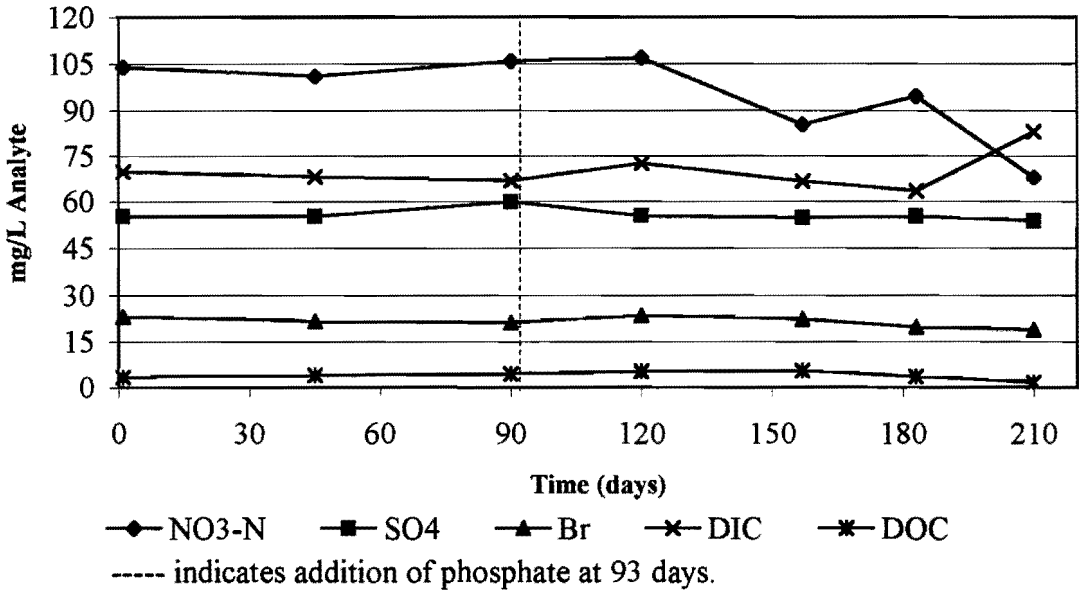
Each depth interval studied showed some denitrification. Observations for each depth include increases in DIC, decreases in NO_3^--N after the addition of phosphate at 93 days, and visible degassing of the solution as denitrification progressed. No conclusions were drawn in regard to the decrease of S^- or SOC throughout the study.

Figure 3 shows the average values of the aqueous analytes over time. The only significant changes in concentrations were an average decrease of 36.1 mg/L NO_3^--N and an average increase of 13.3 mg/L DIC. No changes in SO_4^{-2} concentration were

measured. The lack of change in concentration of SO_4^{-2} was expected because only traces of sulfides were measured at this depth. Heterotrophic activity seemed to be the cause for the denitrification at this depth as evidenced by the increase in DIC.

In-situ, denitrification is not likely to occur in sediments near the water table (2.3-3.2 m) because the oxygen concentration typically is too high to support it; however the isolation of the sediments from oxygen within each sample container allowed some denitrification to occur. The denitrification observed is likely due to the limited oxygen supply in the containers, the presence of anaerobic bacteria capable of using nitrate, and the availability of small amounts of SOC as electron donors.

Figure 3. Average concentration of dissolved analytes over time at 2.3-3.2 meters.



Below the water table, the oxygen concentration would become depleted allowing denitrification to occur. The lack of oxygen below the water table also allows sulfide

minerals to exist without the occurrence of oxidation. Schlag (1999) studied the aquifer in-situ at a depth interval of 4.6-6.1 m and showed that denitrification was occurring naturally at that depth.

Figure 4 shows the average values of the aqueous analytes over time in the sediments collected at the ISM depth (4.9-5.8 m). The significant changes in concentrations were an average decrease of 53.1 mg/L NO_3^--N, an average increase of 24.6 mg/L DIC, and an average increase of 33.6 mg/L SO_4^{-2}. Superficially, it appears as if autotrophic and heterotrophic denitrification is occurring in the samples from this depth interval, evidenced by the increasing DIC and SO_4^{-2}. However, the change in concentration of SO_4^{-2} cannot be wholly attributed to autotrophic processes due to the fact that SO_4^{-2} increased in the control samples as well.

Figure 4. Average concentration of dissolved analytes over time at 4.9-5.8 meters.

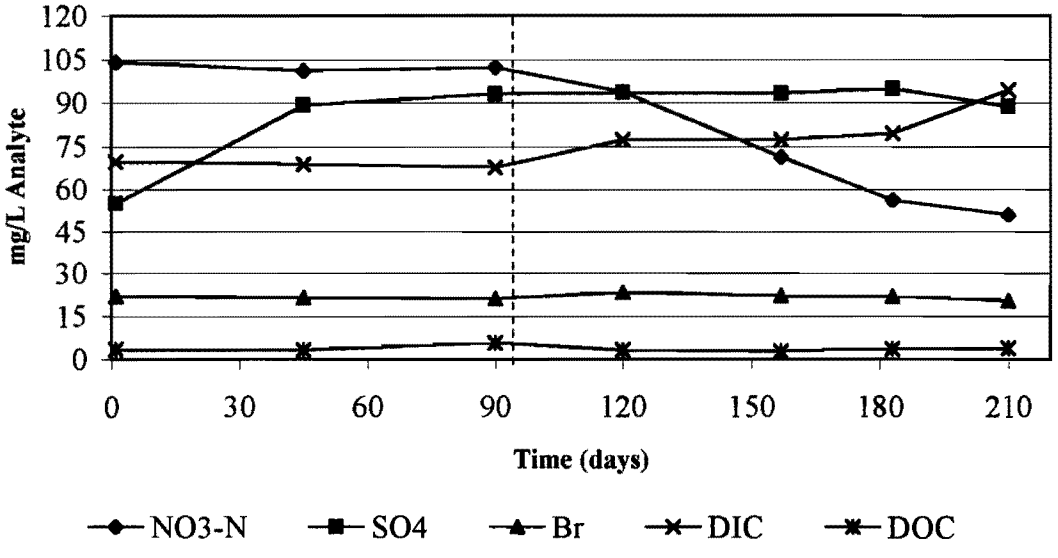
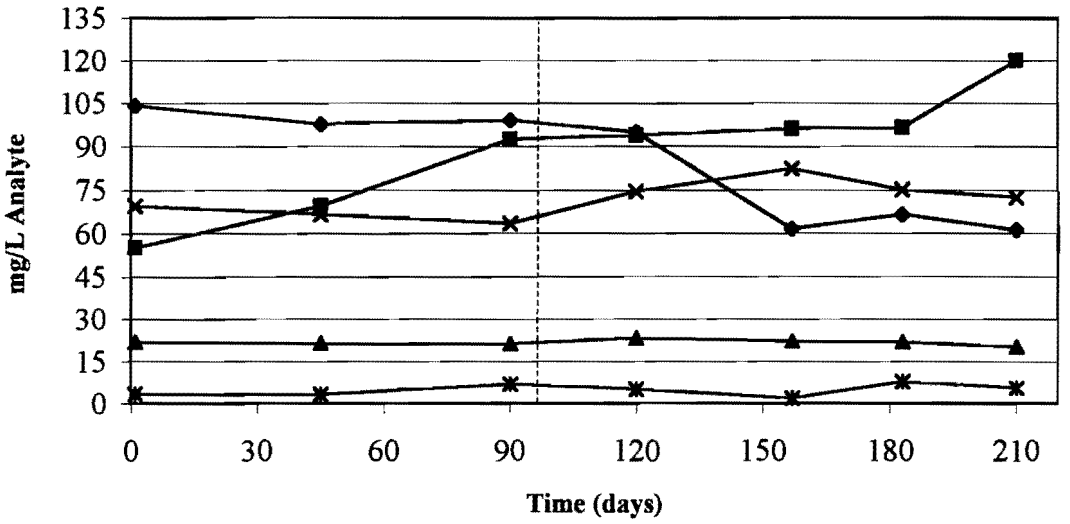


Figure 5 shows the average values of the aqueous analytes over time at 6.7-7.9 m. The only significant changes in concentrations were an average decrease of 42.9 mg/L NO_3^--N and an average increase of 64.9 mg/L SO_4^{-2}. Autotrophic and heterotrophic process appear to be active at this depth interval as well; however, the increasing SO_4^{-2} at this depth interval is suspicious because of similar occurrences in the control sample at this depth. As a result, the increasing SO_4^{-2} cannot be attributed to autotrophic processes.

Figure 5. Average concentration of dissolved analytes over time at 6.7-7.9 meters.



---- indicates addition of phosphate at 93 days.

Electron Donor Analyses

Autotrophic denitrification did not appear to be a major process in the samples for this study. The amount of SO_4^{-2} that was generated was not great enough to allow for any

decrease in the content of sulfides in the sediments to be measured throughout the experiment. At 2.3-3.2 m very little sulfide was present in the sediments at the beginning of the experiment, therefore a measurable change in content would not be expected.

After 210 days of incubation with the amended water, no significant trends were seen regarding a change in the content of electron donors at each depth interval. The content of S^- and SOC content at each depth interval over time can be seen in Figures 6 and 7.

SOC and S^- utilization may not have been detectable at any interval because the weight percent of electron donors in the sediments was not perfectly homogeneous even though the sediments used in the experiment were homogeneous in a bulk state. The total content of SOC and S^- was measured in tenths of a percent by weight and varied slightly from sample to sample. The variable content of these electron donors in each sample container was not anticipated and may have masked any measurable change in their content.

Figure 6. Sulfide content at each depth interval throughout the study.

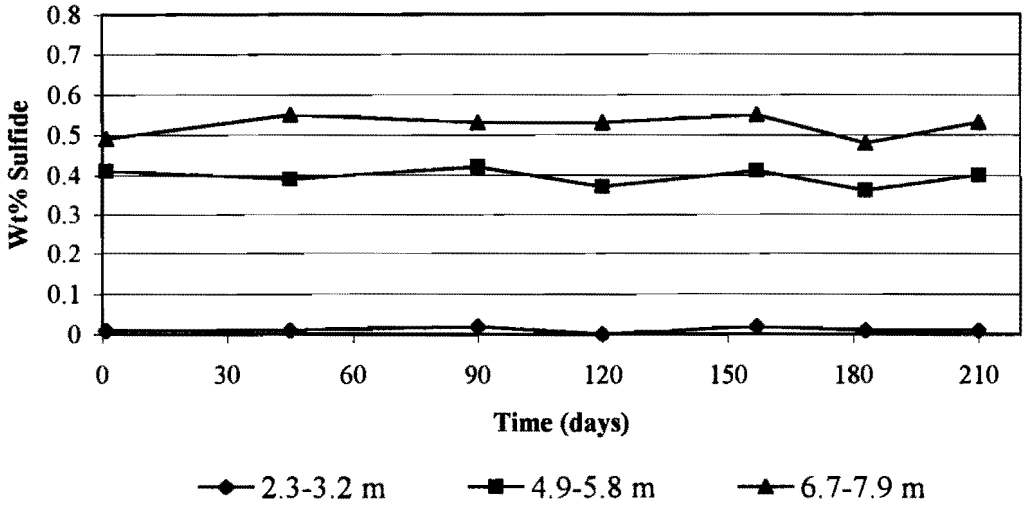
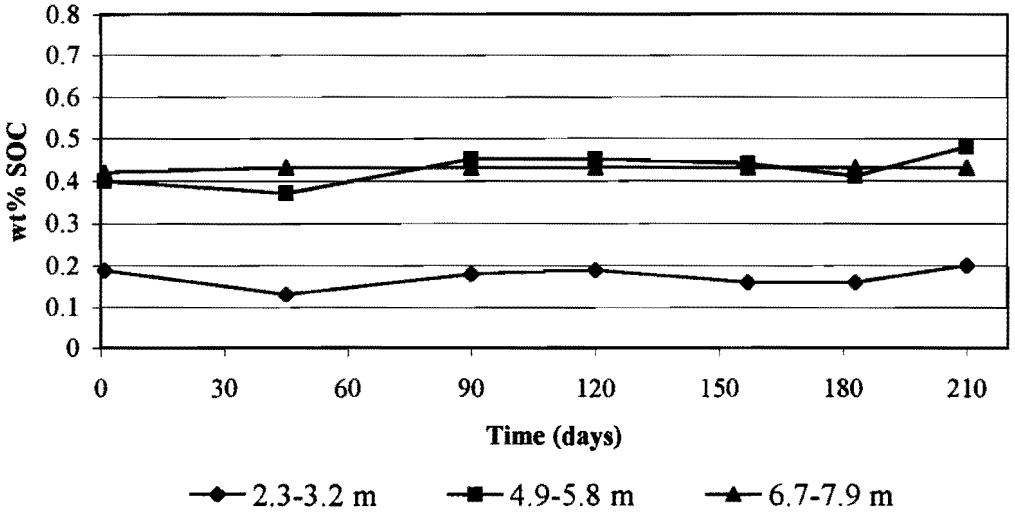


Figure 7. SOC content at each depth interval throughout the study.

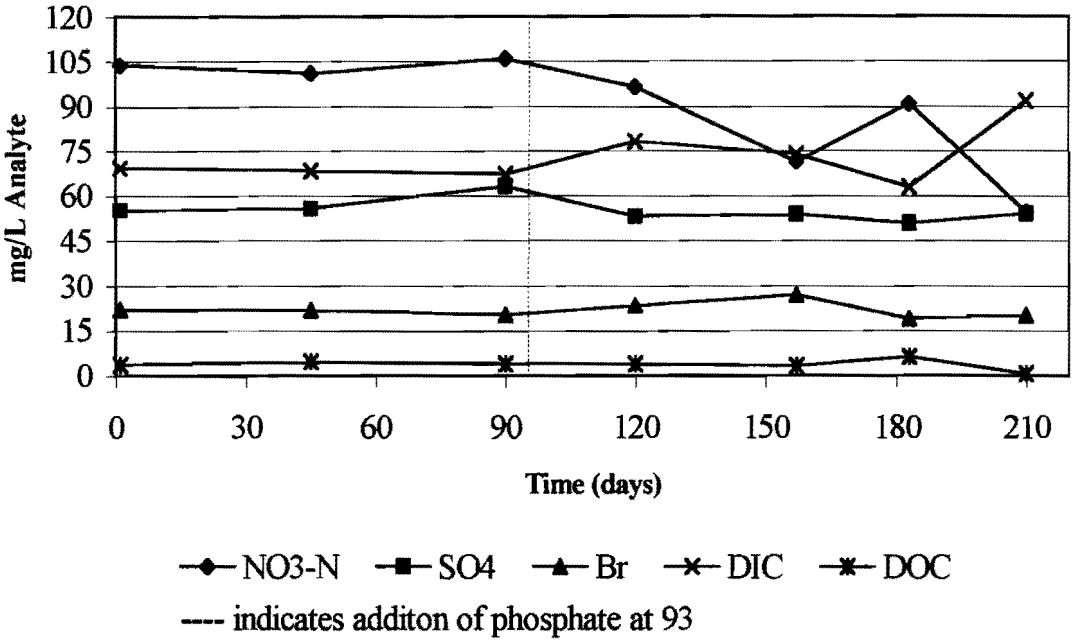


Although heterotrophic processes seemed to be dominant in the experimental samples, there was no detectable change in the content of SOC throughout the experiment. The analytical technique used to determine the quantity of SOC might have been problematic in detecting a change in content. The technique used required that the carbonate fraction of the sediments be removed with acid prior to analysis so that interference between inorganic carbon and organic carbon would not occur. The acid used to remove carbonates was not analyzed for a dissolved fraction of SOC. The SOC left after the acid treatment and measured in the sediments is called acid non-purgeable organic carbon because it does not dissolve. It is possible that the sediments contained types of organic carbon that dissolve in acid and that a detectable change could have occurred in that fraction, but was destroyed with the acid treatment.

Maximum Observed Denitrification

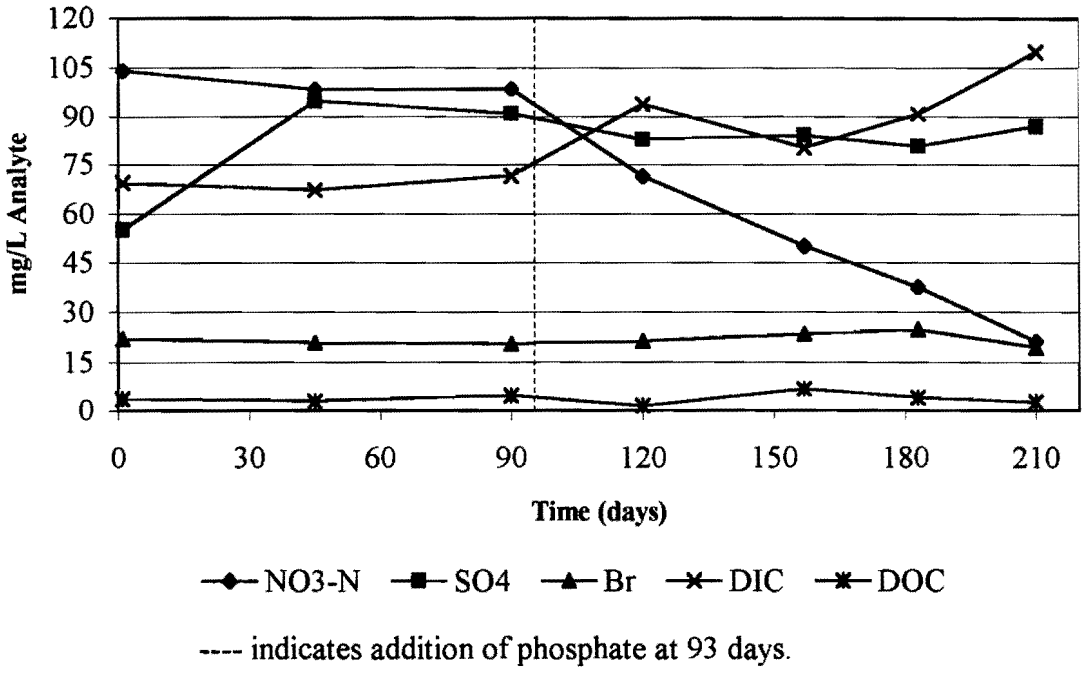
The maximum loss of NO_3^--N at 2.3-3.2 m was 49.3 mg/L (3.52 mmol/L) and was accompanied by an increase in the concentration of DIC of 22.4 mg/L (1.86 mmol/L). The expected increase of DIC when using Reaction (4) would be 52.8 mg/L (4.40 mmol/L). The measured increase of DIC accounts for 42.3% of the denitrification. As previously discussed, DIC was the only by-product of denitrification that was measured at this depth and is an indication of heterotrophic activity. Figure 8 shows the samples that had the greatest amount of denitrification and the associated analytes.

Figure 8. Samples with the most denitrification and associated dissolved components at 2.3-3.2 meters



The maximum loss of NO_3^--N at 4.9-5.8 m was 82.9 mg/L (5.92 mmol/L) and was accompanied by an increase in the concentration of DIC of 40.7 mg/L (3.39 mmol/L). If all of the denitrification was occurring by heterotrophic activity shown by Reaction (4), the expected concentration of DIC would be 88.9 mg/L (7.40 mmol/L). Less than half of the expected concentration of DIC was measured, accounting for 45.8% of the denitrification. Samples that had the most denitrification and their associated analytes at this depth are shown in Figure 9.

Figure 9. Samples with the most denitrification and associated dissolved components at 4.9-5.8 meters.



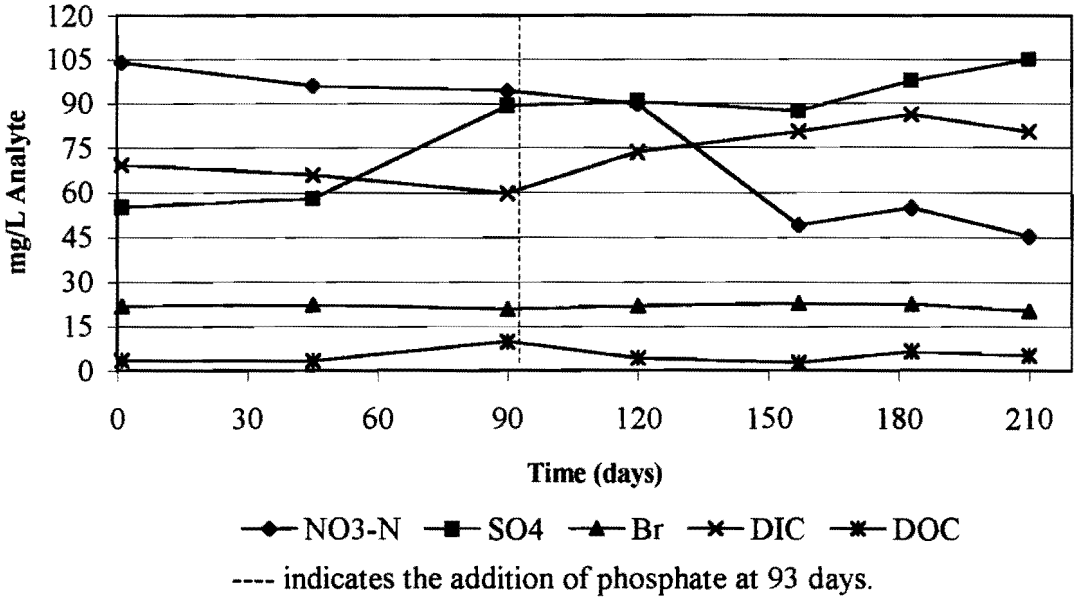
---- indicates addition of phosphate at 93 days.

At 4.9-5.8 m, denitrification also was accompanied by an increase of 31.8 mg/L (0.33 mmol/L) SO_4^{-2}. As discussed previously, this increase cannot be definitively attributed to denitrification, because of the increasing SO_4^{-2} in the control samples. However, even if this increase were due to autotrophic denitrification based on Reaction (3), it would only account for the reduction of 7.00 mg/L NO_3^--N or 8.45% of the total denitrification.

Similar observations were made in samples containing sediments collected at 6.7-7.9 m. The greatest loss of NO_3^--N over 210 days was 58.8 mg/L (4.20 mmol/L) and was accompanied by an increase of 10.9 mg/L (0.91 mmol/L) DIC, as seen in Figure 10. If all of the denitrification was due to heterotrophic activity, the expected increase in DIC

would be 63.1 mg/L (5.25 mmol/L). Only 17.3% of the denitrification is accounted for by the observed increase in DIC. An increase of 49.9 mg/L (0.52 mmol/L) SO_4^{-2} also occurred in these samples accounting for no more than 18.6% of the reduced NO_3^--N. It is likely that at least some, if not all, of the increase in SO_4^{-2} is due to oxidation by oxygen, not nitrate, causing the calculated autotrophic denitrification value to be high. Regardless of how much oxidation occurred via oxygen or nitrate, it is apparent that autotrophic denitrification was not a dominant process in the samples containing sediments from 4.9-5.8 m and those from 6.7-7.9 m.

Figure 10. Samples with the most denitrification and associated dissolved components at 6.7-7.9 meters.



---- indicates the addition of phosphate at 93 days.

At every interval, an increase in the concentration of DIC was observed, indicating heterotrophic activity, but not all of the denitrification can be accounted for with the increase in concentration of DIC. Two possible answers for this are that

denitrification was occurring using other forms of inorganic electron donors that were not identified or that some of the DIC generated was not measured.

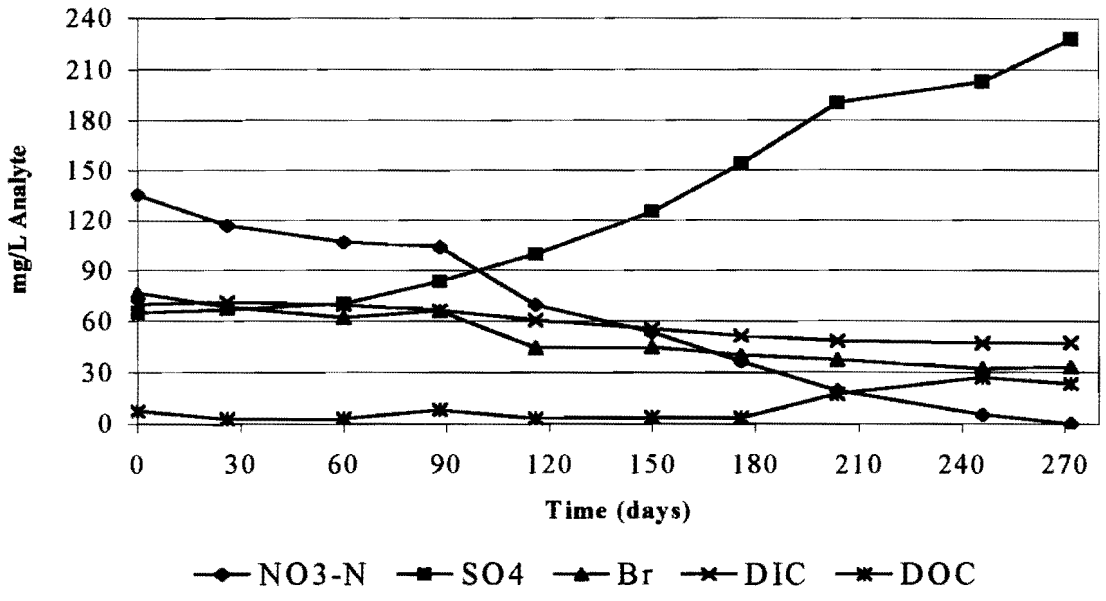
Based on the literature, another inorganic electron donor that could be utilized in denitrification is ferrous iron (Fe^{+2}), as shown in Reaction 2. In this reaction, it takes five moles of Fe^{+2} to reduce every mole of NO_3^-. No measurements of Fe^{+2} were done in the laboratory experiment and its participation in denitrification is not known. However, Postma's (1990) work implies that Fe^{+2} concentrations necessary for significant denitrification are much greater than the Fe^{+2} concentrations measured at the EVA field site (Schlag, 1999). Therefore it is doubtful that Fe^{+2} played an important role as an electron donor for denitrification in the laboratory experiment with EVA sediments.

A more likely explanation for the unaccounted denitrification is that not all of the DIC generated was measured. DIC tends to be unstable in solution. Throughout the study, visible degassing occurred in many sample containers. The escaping gas bubbles may have been CO_2, N_2, or more likely, a combination of the two. The composition of the escaping gas is not known because it was not collected nor analyzed.

Another explanation for low DIC measurements is that it is precipitating out of solution in the form of calcite ($CaCO_3$). The in-situ study (Schlag, 1999) observed a decrease in the concentration of DIC as denitrification progressed, even though some of the denitrification was attributed to heterotrophic activity. Figure 11 shows the decrease in DIC (as HCO_3^-) during the in-situ study, as well as the change in concentration of other analytes. If heterotrophic activity were occurring, an increase in DIC would be

expected. It was determined that calcite was supersaturated in the solution throughout the in-situ study. A precipitate that formed in samples taken from the ISMs was identified using XRD as magnesian calcite. Small amounts of precipitate were also seen forming in the experimental sample containers; however, it could not be separated from the sediments within and was not analyzed for composition. It is possible that both the degassing of DIC as CO_2 and the precipitation of it as carbonate minerals could account for the DIC generated as a result of heterotrophic denitrification but not measured in the samples.

Figure 11. Analytes measured during an in-situ study in the EVA (Schlag, 1999).



The results of depth interval 4.9-5.8 m are important because they can be compared to the results of the ISM study that was conducted at the same site at the same depth. Trends for this study show a decrease in NO_3^--N, DIC, and Br^- and an increase in

SO_4^{-2}, as seen in Figure 11 (Schlag, 1999). After taking dilution into account, it was determined that at least 61% of the denitrification observed was autotrophic. The dominant in-situ process at this depth interval is autotrophic. The lab experiment showed that less than 10% of the denitrification was autotrophic. This finding is important because it shows denitrification experiments done in the lab may not be a good indicator of what actually happens in-situ, as the results of these two studies are not similar.

Overall, the results of the experiment show the laboratory study of denitrification was more complicated than expected. Measuring electron donors in the sediments as well as maintaining proper environmental conditions in the lab were more difficult than anticipated. The results of this experiment did not mimic in-situ results well enough to allow new insights in regard to in-situ electron donor utilization.

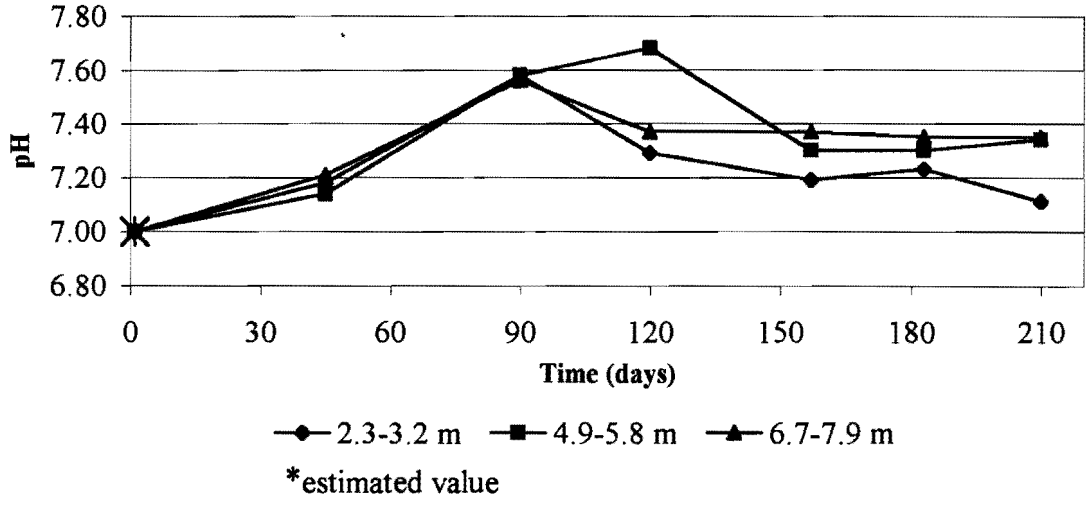
After the experiment, it was postulated that the environmental conditions that bacterial populations function in may be as important as measuring the electron donors available to them. Environmental factors that may affect bacterial populations include oxygen concentration, pH and nutrient availability (Chapelle, 2001).

The inability to control oxygen contamination in the samples seemed to have a major affect on this study. Efforts were made to keep all samples in a nitrogen atmosphere, but oxygen contamination occurred. Keeping the sediments oxygen-free may have been critical in sustaining the obligate anaerobic populations of autotrophic and heterotrophic bacteria.

No attempts were made to identify or classify the types of bacteria present in the samples. The population of heterotrophs present in the EVA may be facultative and more tolerant of oxygen exposure than autotrophs. Overall, heterotrophic denitrification was the dominant process during the laboratory experiment compared to the in-situ study (Schlag, 1999), where autotrophic processes dominated. The apparent reasons for this difference may lie in the type of bacterial populations that flourished in each case.

One environmental parameter that probably did not change significantly enough to affect the bacterial population was the pH of the water. The pH values throughout the lab study ranged between 7.1 to 7.6. Figure 12 shows the average pH values measured at each interval throughout the study. Bacteria are capable of living in a wide range of pH values and usually maintain an intercellular pH around 7.5 (Chapelle, 2001). It is not likely that the pH affected the outcome of the study significantly with regard to the type of denitrification occurring in the experimental samples.

Figure 12. pH at each depth interval.



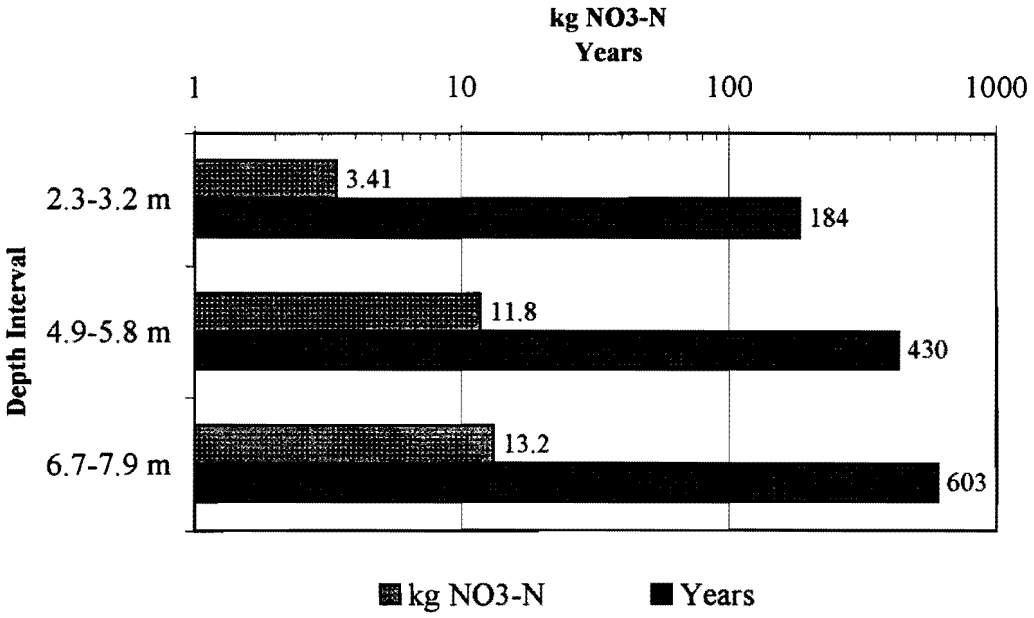
A factor that did affect the outcome of the experiment was the addition of phosphate to the sample containers on day 93. In nature, groundwater flow and sediments dissolution provides a fresh supply of nutrients to microbes on the sediments. Nutrients essential to microbes include carbon, nitrogen, phosphorus, potassium, sulfur, and smaller quantities of iron, calcium, magnesium and chloride. If any of the nutrients are limited relative to carbon, microbial growth may also be limited (Chapelle, 2001; National Research Council, 1993). Nutrients necessary for microbial growth were either provided by the addition of chemicals to the water or can be found in the mineral phases present in the sediments, with the exception of phosphate. The addition of phosphate at 93 days stimulated the samples into a faster rate of denitrification. Coincident with denitrification was an increase in the concentration of DIC, a by-product of nitrate reduction (see Reaction 4) and heterotrophic activity.

Maximum Denitrification Potential

Calculations were done to determine the maximum denitrification potential of the EVA sediments at this site based on the electron donors present. The calculations were done using 1 cubic meter of aquifer material with an estimated 30% porosity and an average sediment density estimated to be 2650 kg/m^3. The mass of sediment in 1 cubic meter equals 1855 kg. Water accounts for 300 L, approximately equal to 300 kg. The weight percent of electron donors was determined in the lab (see Figure 1) and used to calculate the mass of electron donors present in the sediment portion of 1 cubic meter of

the EVA. Figure 13 shows the denitrification potential in one cubic meter of the EVA study site sediments at each depth interval.

Figure 13. Denitrification potential based on electron donor content and the average rate measured in the lab at each depth interval.



At 2.3-3.2 m the average measured S^- concentration was converted to pyrite and used in Reaction (3). Pyrite equals ~0.02 wt% in this interval, equal to approximately 347 grams (2.89 mols) of FeS_2, which is capable of reducing 121.5 grams (8.67 mols) NO_3^--N. The SOC content of the EVA at this interval average 0.19 % by weight, equal to 3.52 kg (293.4 mols) in 1 cubic meter. The amount of NO_3^--N that can be reduced using Reaction (4) with SOC is 3.3 kg (234.7 mols). Adding the amount of NO_3^--N from both sources of electron donors that were measured, 1 cubic meter of the EVA at the field site can attenuate 3.41 kg NO_3^--N. During the experiment, the average rate of denitrification

over the 210 days was 0.17 mg/L/day. At this rate, the EVA could continue to denitrify over a period of 184 years.

The maximum potential of denitrification is strictly based on the quantity of electron donors present at this depth. Using the quantity of electron donors as a gauge for maximum denitrification potential is not appropriate in this instance because the environmental conditions are not present to support it (Korom, 1992; Mayer, 1992). As previously noted, sediments from near the water table would not be able to reduce NO_3^--N because the concentration of oxygen would be too high.

At 4.9-5.8 m, the average content of S^- is 0.40% by weight and converts to 0.75% by weight FeS_2. In a cubic meter of EVA at this depth interval there is 13.9 kg (115.8 mols) FeS_2. SOC averages 0.48% by weight equal to 8.90 kg (741.0 mols). 4.86 kg (347.0 mols) NO_3^--N can be reduced by pyrite (Reaction 3) and 6.92 kg (494.0 mols) NO_3^--N can be reduced utilizing SOC (Reaction 4). The total NO_3^--N load would equal 11.78 kg. It would take 430 years to attenuate 11.78 kg NO_3^--N at the rate of 0.25 mg/L/day, the average rate measured throughout the lab experiment at this interval.

At 6.7-7.9 m the average measured S^- concentration equals 0.49 weight %. The FeS_2 equivalent is 0.92 weight % equal to 17.1 kg (142.5 mols) in one cubic meter, capable of reducing 5.94 kg (424.1 mols) NO_3^--N. The SOC content of the EVA at this interval average 0.42 weight %, equal to 7.79kg (648.6 mols) in 1 cubic meter. The amount of NO_3^--N that can be reduced using Reaction (4) with SOC is 7.27kg (519.0 mols). Adding the amount of NO_3^--N from both sources of electron donors, 1 cubic

meter of the EVA can attenuate 13.2 kg (942.4 mols). NO_3^--N. During the experiment, the average rate of denitrification over the 210 days was 0.20 mg/L/day. At this rate, the EVA could continue to denitrify over a period of 603 years.

The average rate of denitrification measured in-situ by Schlag (1999) with a tracer test was 0.22 mg/L/day. A second tracer test conducted by Skubinna (University of North Dakota, personal communication) in the same set of ISMs at the same field site shows that rate of denitrification was cut in half to 0.11 mg/L/day. This suggests that the availability of electron donors present within the sediments of the EVA at this site may be limited to the bacteria that use them in denitrification. Based on the observation of decreasing rate, it is not likely that the EVA has the denitrification potential that is calculated based on the quantity of electron donors at this or other depth intervals.

CONCLUSIONS

This study was based on the observation that autotrophic denitrification with sulfide as an electron donor is a dominant in-situ process at the EVA field site (Schlag, 1999). The expected results of the laboratory study were that autotrophic denitrification would be reasonably duplicated in the lab accompanied by a measurable decrease in the sulfide minerals serving as electron donors for the reaction. However, the lab results indicated that heterotrophic denitrification was the dominant nitrate reducing process for this experiment. Because of the heterotrophic nature of the denitrification, no decrease in sulfide content was measured; however, the quantity and major species of sulfide (pyrite) present in the EVA were determined. Knowledge gained from this study gives insight to the complexity of denitrification and shows that studies done in the lab are not necessarily a good gauging technique to determine the denitrification capabilities of an aquifer.

It was determined that measuring the quantity of electron donors using this technique does not give insight to the denitrification rates of the EVA. The theoretical calculations done for one cubic meter of aquifer material suggests that the availability of the aquifer to utilize all of the electron donors is not very likely. If the calculated

potentials were near reality, it would be possible to perform multiple in-situ tracer tests in the ISMs with no change in rate of denitrification observed for many years.

Recommendations for Future Work

Insights gained in this study open the door for further research to be continued the EVA and on the topic of denitrification. Four ideas for further study came to mind throughout this study. One idea is to identify the microbial populations responsible for denitrification in the EVA and the required environmental conditions in which the microbes thrive. The identification of subsurface populations could determine factors important to denitrification rates such as metabolism and bacterial growth rates. Environmental conditions would be important in identifying whether autotrophs or heterotrophs are dominant in the subsurface.

Another idea is to study sediments isolated in a column designed to allow better control of environmental parameters. Using a column to study the utilization of electron donors may be a way to prevent contamination of sediment with oxygen resulting in the killing off of essential microbes. This would also allow for a more realistic environmental setting to be achieved. Factors such as porosity, temperature, and even pressure could be controlled in a lab column study.

A third idea for further investigation is to determine the quality of the organic matter and the crystallinity of the sulfides present in the aquifer. This study showed that the amount of the electron donors present in the sediments is not a good gauging

technique for determining the denitrification potential of an aquifer. The quality and availability of the electron donors could be studied in order to develop a method to characterize an aquifer's denitrification potential.

The final idea is to develop a method to collect sediments from within the ISMs during an in-situ study to determine the utilization of electron donors in a field setting. If sediment samples could be collected throughout a tracer test, there would be no need to attempt bringing samples into a lab setting for denitrification experiments of a similar nature. Electron donor utilization data could be gained along with the ISM study, making data sets much more complete.

APPENDIX A

ADJUSTMENTS MADE TO SOLID ORGANIC CARBON ANALYSIS TO ACCOUNT FOR LOSS OF CARBONATE MINERALS

The SOC measurements were adjusted to take into account the amount of carbonate that was leached from the sample prior to analysis. This adjustment was done by adding the average content of carbonate removed from the original sediment to the weight of the sample analyzed. The calculations used in the adjustment are as follows:

Weight SIC removed = Wt Fraction SIC Removed * Sample weight

Weight SOC = Weight Fraction SOC (Sample wt - wt SIC removed)

Adjusted OC wt% = (wt SOC/Sample weight) * 100%

Example:

Sample weight = 900.0 mg

Weight% SIC removed = 15.2% = 0.152

Wt% SOC = 0.285% = 0.00285

1X - 0.152X = 900.0 mg

X = 900.0/0.848 mg = 1061.3 mg

SOC weight = 900.0 mg x 0.00285 = 2.565 mg

Adjusted OC wt% = (2.565/1061.3 mg) x 100% = 0.242% SOC

APPENDIX B

XRD INFORMATION AND SCANS

XRD analysis was done at the Materials Characterization Lab at North Dakota State University, Fargo, North Dakota. The scans were analyzed using the database at NDSU found in JADE 3.1 (Materials Data Inc, 1997). Minerals and mineral groups identified in the EVA include quartz (SiO_2), potassic feldspar (example - microcline $KAlSi_3O_8$), plagioclase feldspar (albite - anorthite solid solution series $NaAlSi_3O_8$ - $CaAl_2Si_2O_8$), calcite ($CaCO_3$), dolomite ($CaMg(CO_3)_2$), smectite (group of minerals, example - montmorillonite $[(Al_{1.77}Fe^{3+}_{0.03}Mg_{0.20})(Si_{3.74}Al_{0.26})O_{10}(OH)_2]$), hydrated silica ($SiO_2 \cdot nH_2O$), mica-illite ($K_{0.6}Mg_{0.25}Al_{2.3}Si_{3.5}O_{10}(OH)_2$), kaolinite ($Al_2Si_2O_5(OH)_4$), amphibole (example - hornblende $(Ca,Na)_{2-3}(Mg,Fe^{2+},Fe^{3+},Al)_5(Al,Si)_8O_{22}(OH)_2$) and pyrite ($FeS_2$) (Faure 1998; Motanna et al., 1978).

Figure 14 is the scan of sediments collected at the interval of 2.3-3.2 m below the surface, Figure 15 is the scan of minerals found at 4.9-5.8 m below the surface, and Figure 16 is the scan of minerals found at 6.7-7.9 m below the surface.

Figure 14. XRD scan of sediments collected at 2.3-3.2 meters.

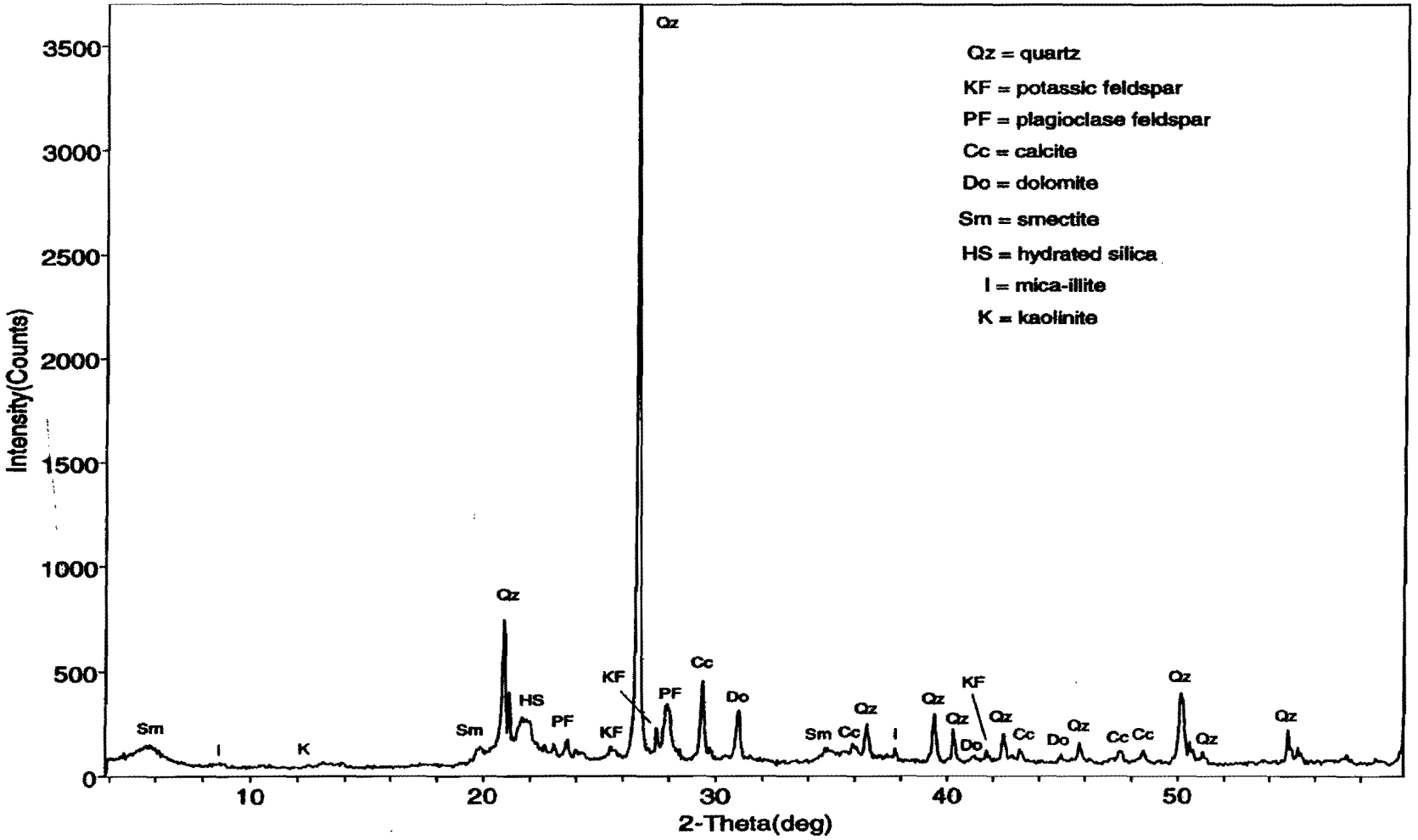


Figure 15. XRD scan of sediments collected at 4.9-5.8 meters.

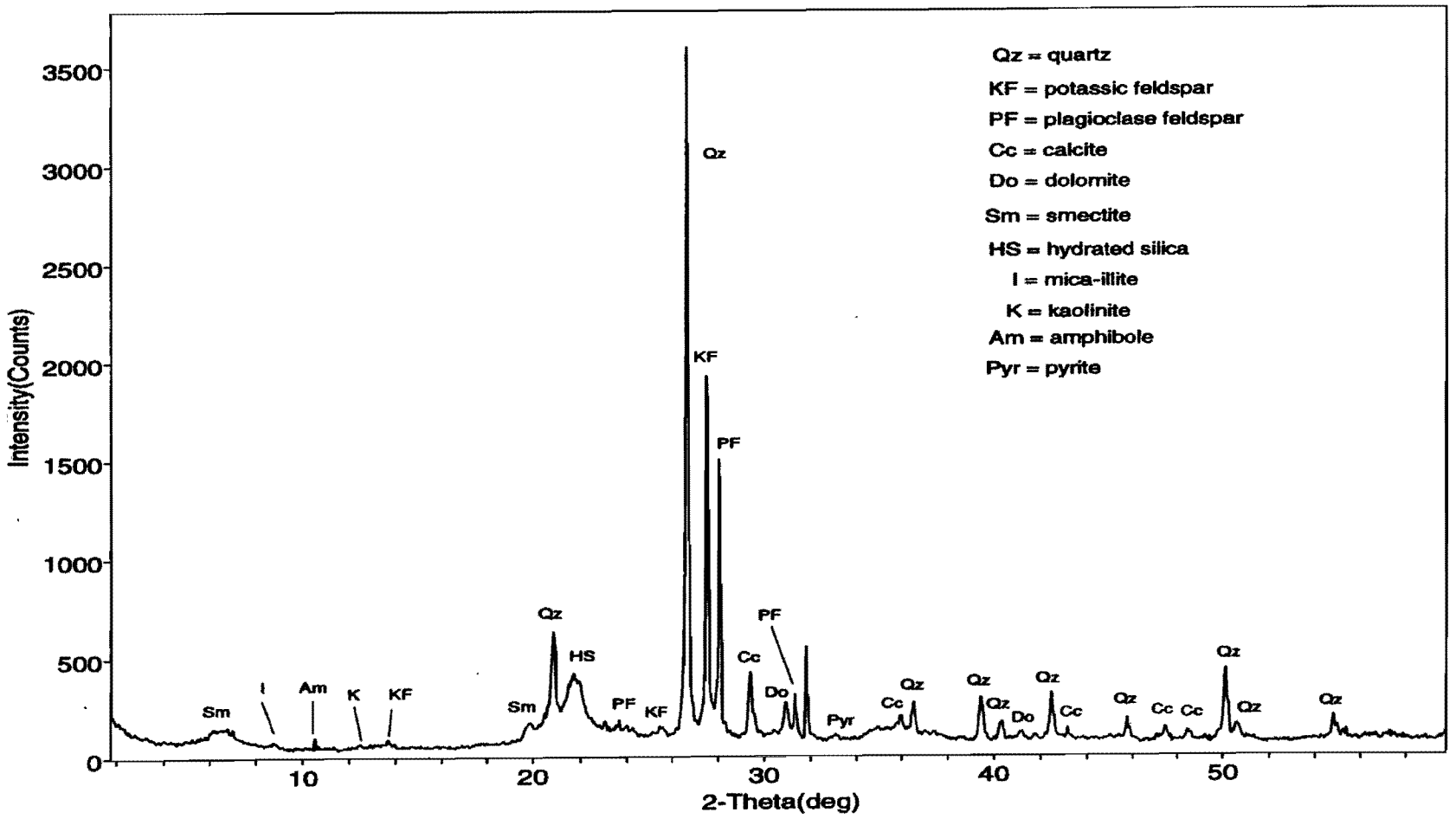
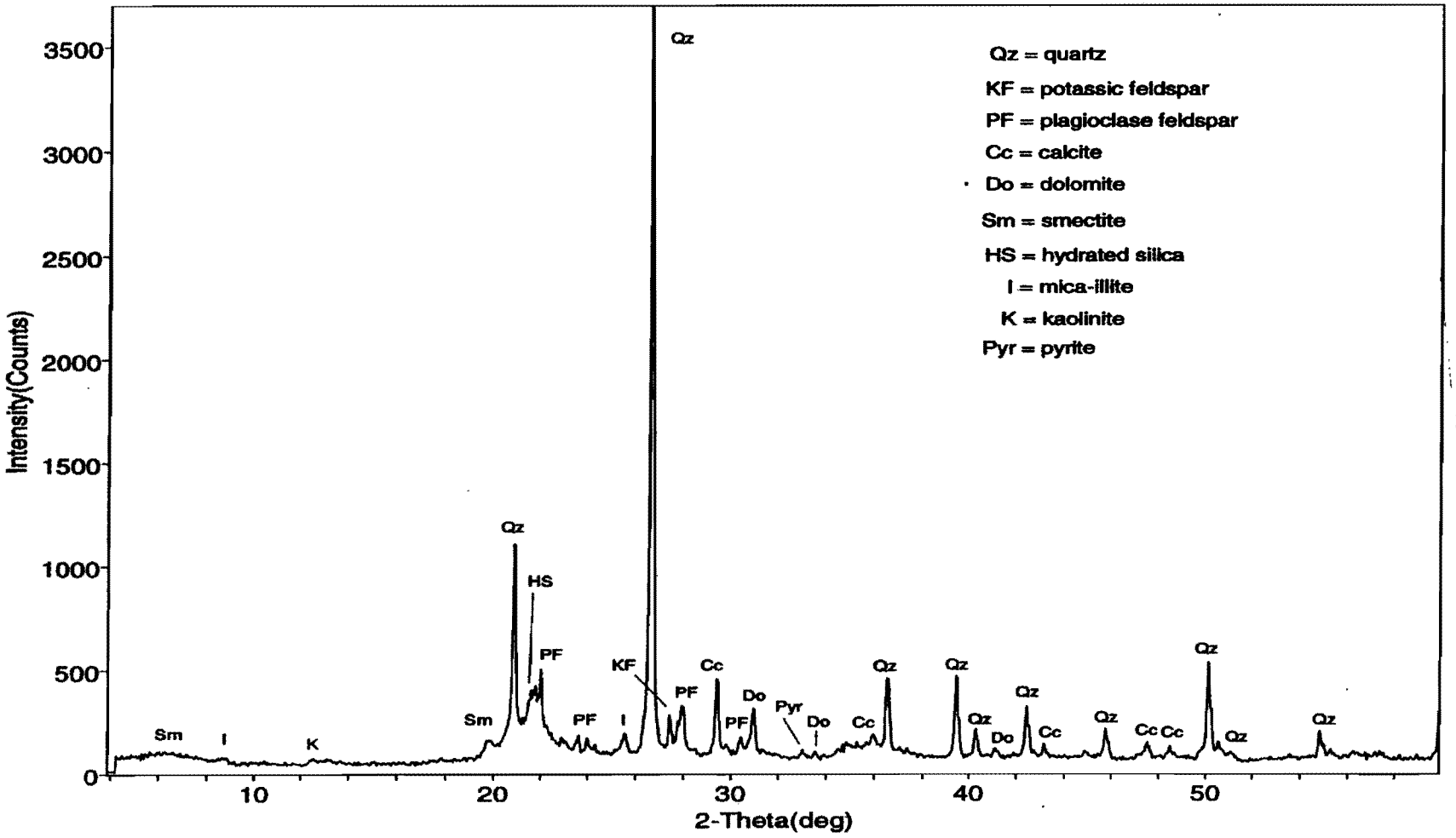


Figure 16. XRD scan of sediments collected at 6.7-7.9 meters.



APPENDIX C

ANALYSES DATA, AVERAGES AND

STANDARD DEVIATION TABLES

Table 2. Data collected from depth intervals throughout the study period.

Sample ID*	Time	NO_3^--N	SO_4^{2-}	Br^-
meters	days	mg/L	mg/L	mg/L
Amended				
water	1	104/104	55.1/55.1	21.9/21.9
2.3-3.2	45	101/101/101	55.9/55.7/53.7	21.6/21.5/21.2
2.3-3.2	90	106/107/106	63.2/57.9/58.2	20.4/20.4/19.9
2.3-3.2	120	112/112/96.4	55.0/57.9/53.2	22.7/23.8/23.4
2.3-3.2	157	92.5/91.8/71.5	54.5/55.4/54.0	19.1/20.2/26.9
2.3-3.2	183	97.7/90.9/95.3	52.8/50.8/53.2	20.0/18.9/19.8
2.3-3.2	210	54.7/74.5/74.4	54.2/53.5/53.2	20.0/19.0/17.3
Formation				
water	1	nd	56.7	nd
Ctrl 2.3-3.2	45	nd	57.9	nd
Ctrl 2.3-3.2	90	nd	62.5	nd
Ctrl 2.3-3.2	120	nd	57.4	nd
Ctrl 2.3-3.2	157	nd	61.0	nd
Ctrl 2.3-3.2	183	nd	55.2	nd
Ctrl 2.3-3.2	210	nd	58.6	nd

Table 2. Continued

Sample ID* meters	Time days	TDC mg/L	DIC mg/L	DOC mg/L	pH	DO mg/L
Amended water	1	72.9/72.8	69.3/69.8	3.68/3.03	nr	nr
2.3-3.2	45	73.1/71.8/71.4	68.5/67.8/68.0	4.65/3.99/3.42	7.16/7.17/7.20	3.93/4.19/3.73
2.3-3.2	90	70.8/71.1/70.9	67.2/67.0/65.9	4.04/4.98	7.55/7.59/7.60	2.47/2.91/1.30
2.3-3.2	120	83.7/67.5/82.1	79.9/59.3/78.1	3.82/8.22/3.98	7.24/7.36/7.28	1.84/3.77/0.09
2.3-3.2	157	68.0/70.7/77.2	63.0/62.7/73.9	5.02/8.04/3.23	7.14/7.19/7.25	0.05/0.07/0.08
2.3-3.2	183	64.0/69.4/67.2	62.7/63.0/64.3	1.31/6.42/2.96	7.32/7.17/7.20	2.48/0.04/0.05
2.3-3.2	210	92.4/84.6/77.1	91.7/81.9/75.1	0.68/2.69/1.95	6.96/7.19/7.19	0.08/0.06/0.07
Formation water	1	74.0	71.1	3.01	nr	nr
Ctrl 2.3-3.2	45	74.9	71.6	3.27	7.24	3.44
Ctrl 2.3-3.2	90	73.8	70.3	3.54	7.63	4.24
Ctrl 2.3-3.2	120	74.1	71.0	3.10	7.49	4.54
Ctrl 2.3-3.2	157	66.7	64.7	2.02	7.45	5.09
Ctrl 2.3-3.2	183	66.4	61.8	4.64	7.48	3.34
Ctrl 2.3-3.2	210	65.4	61.4	4.05	7.54	6.35

Table 2. Continued

Sample ID*	Time	SOC**	SIC	S^-	Sediment	Solution
meters	days	wt%	wt%	wt%	grams	mL
2.3-3.2	1	0.18/0.19/0.19	nr	0.01/nd/0.01	na	na
2.3-3.2	45	0.13/0.11/0.14	nr	0.01/0.01/nd	47.7/57.3/69.9	481/464/479
2.3-3.2	90	0.25/0.16/0.11	nr	0.02/0.01/0.03	60.0/64.3/66.7	436/445/434
2.3-3.2	120	0.17/0.18/0.16	nr	nd/0.01/nd	37.2/66.9/55.7	448/423/445
2.3-3.2	157	0.17/0.14/0.16	15.4/14.4/14.6	0.02/0.02/0.01	52.1/60.4/88.6	444/440/436
2.3-3.2	183	0.16/0.16/0.16	13.9/14.0/14.6	0.01/0.01/0.02	53.6/54.1/62.0	445/447/442
2.3-3.2	210	0.24/0.18/0.18	15.2/12.4/13.0	nd/0.01/0.03	nr/50.9/74.4	nr/453/442
Ctrl 2.3-3.2	210	0.17	14.8	0.01	87.2	890

* Sample ID indicates the depth interval sediments were collected at.
** Weight % adjusted to account for the weight loss that occurred due to leaching of carbonate minerals.
1/2/3 indicates analysis of three separate sample containers within the set.
nr indicates data not recorded
nd indicates not detectable
na indicates not applicable

Table 2. Continued

Sample ID* meters	Time days	NO_3^--N mg/L	$SO4^{2-}$ mg/L	Br^- mg/L
Amended water	1	104/104	55.1/55.1	21.9/21.9
4.9-5.8	45	98.4/102/103	94.7/81.5/90.9	20.8/22.0/21.7
4.9-5.8	90	98.7/106/102	90.9/102/86.1	20.3/20.7/21.9
4.9-5.8	120	71.3/93.4/109	82.8/87.2/110	21.1/24.8/24.0
4.9-5.8	157	91.0/72.9/50.1	94.1/101/84.1	21.2/21.5/23.4
4.9-5.8	183	37.6/45.6/85.3	80.6/91.5/112	24.9/19.9/20.8
4.9-5.8	210	21.1/85.3/46.3	86.9/91.8/87.3	19.4/21.1/20.0
Formation water	1	nd	56.7	nd
Ctrl 4.9-5.8	45	nd	75.3	nd
Ctrl 4.9-5.8	90	nd	88.6	nd
Ctrl 4.9-5.8	120	nd	95.0	nd
Ctrl 4.9-5.8	157	nd	105	nd
Ctrl 4.9-5.8	183	nd	102.0	nd
Ctrl 4.9-5.8	210	nd	104.0	nd

Table 2. Continued

Sample ID* meters	Time days	TDC mg/L	DIC mg/L	DOC mg/L	pH	DO mg/L
Amended water	1	72.9/72.8	69.3/69.8	3.68/3.03	nr	nr
4.9-5.8	45	69.9/73.8/72.0	67.1/70.4/68.8	2.86/3.46/3.29	7.16/7.12/7.14	0.13/0.13/0.27
4.9-5.8	90	76.1/70.0/73.8	71.5/62.4/68.8	4.59/7.67/4.98	7.63/7.49/7.62	0.06/0.151.05
4.9-5.8	120	95.1/76.6/69.5	93.8/72.9/65.0	1.34/3.71/4.47	7.46/7.42/7.27	0.08/0.07/0.03
4.9-5.8	157	70.4/82.6/86.8	69.8/81.5/80.2	0.69/1.13/6.56	7.29/7.38/7.23	0.04/0.08/0.12
4.9-5.8	183	83.0/89.7/59.7	90.7/86.2/61.0	nd/3.55/nd	7.22/7.40/7.27	0.11/0.05/0.03
4.9-5.8	210	113/77.0/105	110/73.3/99.4	2.60/3.78/5.43	7.24/7.34/7.44	0.04/0.07/0.04
Formation water	1	74.0	71.1	3.01	nr	nr
Ctrl 4.9-5.8	45	75.8	71.2	4.58	7.04	0.02
Ctrl 4.9-5.8	90	73.3	65.1	8.23	7.54	0.11
Ctrl 4.9-5.8	120	70.5	67.1	3.45	7.33	2.46
Ctrl 4.9-5.8	157	56.9	55.7	1.19	7.92	3.44
Ctrl 4.9-5.8	183	59.1	55.0	4.15	7.61	4.00
Ctrl 4.9-5.8	210	56.5	52.7	3.84	7.62	2.80

Table 2. Continued

Sample ID* EVA(meters)	Time days	SOC** wt%	SIC wt%	S^{-} wt%	Sediment grams	Solution mL
4.9-5.8	1	0.45/0.51/0.32	nr	0.42/0.43/0.39	na	na
4.9-5.8	45	0.26/0.40/0.44	nr	0.40/0.39/0.38	51.1/18.7/16.8	461/486/480
4.9-5.8	90	0.48/0.46/0.42	nr	0.43/0.42/0.40	34.0/26.2/34.1	456/451/452
4.9-5.8	120	0.53/0.40/0.41	nr	0.35/0.38/0.39	34.2/27.8/40.6	462/465/450
4.9-5.8	157	0.43/0.45/0.44	14.7/13.6/13.7	0.43/0.38/.041	33.3/25.2/20.6	462/458/468
4.9-5.8	183	0.43/0.38/0.42	12.5/13.4/13.0	0.37/0.36/0.35	23.2/48.5/48.4	479/439/449
4.9-5.8	210	0.50/0.45/0.48	11.2/11.6/12.2	0.37/0.40/0.43	26.1/24.2/17.4	462/470/465
Ctrl 4.9-5.8	210	0.45	13.8	0.29	16	900

* Sample ID indicates the depth interval sediments were collected.
** Weight % adjusted to account for the weight loss that occurred due to leaching of carbonate minerals.
1/2/3 indicates analysis of three separate sample containers in the same set.
nr indicates data not recorded
nd indicates not detectable
na indicates not applicable

Table 2. Continued

Sample ID* meters	Time days	NO_3^--N mg/L	SO_4^{2-} mg/L	Br^- mg/L
Amended water	1	104/104	55.1/55.1	21.9/21.9
6.7-7.9	45	98.8/98.8/95.9	72.6/77.8/57.9	21.7/20.6/22.3
6.7-7.9	90	101/102/94.1	99.0/90.0/88.9	20.0/22.8/20.8
6.7-7.9	120	89.7/92.6/103	90.5/87.3/104	21.8/22.7/25.0
6.7-7.9	157	49.1/70.4/65.1	87.3/100/101	22.7/20.1/23.3
6.7-7.9	183	81.5/54.9/63.2	78.3/97.7/114	22.0/22.5/21.3
6.7-7.9	183	45.2/59.2/79.0	105/113/142	20.0/19.0/21.1
Formation water	1	nd	56.7	nd
Ctrl 6.7-7.9	45	nd	77.6	nd
Ctrl 6.7-7.9	90	nd	107.4	nd
Ctrl 6.7-7.9	120	nd	123	nd
Ctrl 6.7-7.9	157	nd	139	nd
Ctrl 6.7-7.9	183	nd	159.0	nd
Ctrl 6.7-7.9	183	nd	188	nd

Table 2. Continued

Sample ID* meters	Time days	TDC mg/L	DIC mg/L	DOC mg/L	pH	DO mg/L
Amended water	1	72.9/72.8	69.3/69.8	3.68/3.03	nr	nr
6.7-7.9	45	68.8/71.9/70.8	65.7/68.5/65.7	2.86/3.46/3.29	7.15/7.23/7.24	0.15/0.07/0.13
6.7-7.9	90	68.3/73.3/69.6	61.7/69.1/59.8	6.61/4.15/9.79	7.52/7.57/7.60	0.03/0.04/0.04
6.7-7.9	120	77.6/85.1/75.5	73.4/79.0/71.0	4.21/6.12/4.57	7.41/7.41/7.29	0.04/0.05/0.04
6.7-7.9	157	83.0/83.4/86.2	80.3/82.3/84.4	2.64/1.18/1.80	7.37/7.36/7.38	0.09/0.07/0.08
6.7-7.9	183	77.4/92.3/79.0	66.0/86.0/73.6	11.3/6.37/5.38	7.39/7.31/7.34	0.06/0.04/0.07
6.7-7.9	183	85.3/81.6/66.8	80.2/76.1/61.3	5.06/5.48/5.45	7.41/7.35/7.30	0.08/0.07/0.04
Formation water	1	74.0	71.1	3.01	nr	nr
Ctrl 6.7-7.9	45	74.8	71.0	3.82	7.13	0.05
Ctrl 6.7-7.9	90	69.1	63.7	5.44	7.38	0.19
Ctrl 6.7-7.9	120	66.6	62.9	3.74	7.24	1.31
Ctrl 6.7-7.9	157	43.5	42.1	1.40	7.86	2.53
Ctrl 6.7-7.9	183	43.4	39.9	3.57	7.46	3.62
Ctrl 6.7-7.9	183	35.5	31.1	4.45	8.04	4.32

Table 2. Continued

Sample ID*	Time	SOC**	SIC	S^{-}-S	Sediment	Solution
meters	days	wt%	wt%	wt%	grams	mL
6.7-7.9	1	0.41/0.40/0.44	nr	0.48/0.50/0.49	na	na
6.7-7.9	45	0.43/0.39/0.44	nr	0.53/0.55/0.58	60.7/38.9/45.6	467/494/474
6.7-7.9	90	0.43/0.48/0.37	nr	0.54/0.53/0.51	58.8/33.3/59.7	444/475/430
6.7-7.9	120	0.45/0.39/0.41	nr	0.54/0.53/0.53	67.4/30.7/29.9	440/469/446
6.7-7.9	157	0.43/0.40/0.44	14.7/14.3/13.8	0.56/0.54/0.56	42.2/41.3/31.8	459/448/441
6.7-7.9	183	0.37/0.38/0.51	13.6/14.4/13.8	0.49/0.46/0.49	23.6/31.9/32.3	465/450/448
6.7-7.9	210	0.43/0.45/0.40	11.6/13.4/13.0	0.54/0.51/0.54	73.1/55.0/47.5	518/436/458
Ctrl 6.7-7.9	210	0.4	14.5	0.40	38.1	950

* Sample ID indicates the depth interval sediments were collected.
** Weight % adjusted to account for the weight loss that occurred due to leaching of carbonate minerals.
1/2/3 indicates analysis of three separate sample containers within the set.
nr indicates data not recorded
nd indicates not detectable
na indicates not applicable

Table 2. Continued

Sample ID	Time	NO_3^--N	SO_4^{2-}	Br^-
	days	mg/L	mg/L	mg/L
Formation water	1	nd	56.7	nd
Ctrl H2O	45	107	56.8	21.7
Ctrl H2O	90	107	65.6	29.4
Ctrl H2O	120	104	53.9	24.0
Ctrl H2O	157	104	58.3	23.6
Ctrl H2O	183	104	53.1	23.8
Ctrl H2O	210	103	55.1	18.3

Table 2. Continued

Sample ID	Time	TDC	DIC	DOC	pH	DO
	days	mg/L	mg/L	mg/L		mg/L
Formation water	1	74.0	71.1	3.01	nr	nr
Ctrl H2O	45	76.2	72.7	3.53	7.72	4.83
Ctrl H2O	90	76.6	71.8	4.76	8.08	5.79
Ctrl H2O	120	75.9	72.3	3.67	7.93	4.54
Ctrl H2O	157	60.9	55.9	7.05	7.97	3.55
Ctrl H2O	183	69.9	65.6	4.30	7.94	4.82
Ctrl H2O	210	69.6	66.2	3.37	7.96	6.87

No averages available for this data.
nr indicates data not recorded
nd indicates not detectable
na indicates not applicable

Table 3. Averages and standard deviations for data at each depth interval.

Sample ID*	Time	NO_3^--N		SO_4^{2-}		Br^-	
meters	days	mg/L	S.D.	mg/L	S.D.	mg/L	S.D.
Amended							
water	1	104	0.0	55.1	0.0	21.9	0.0
2.3-3.2	45	101	0.0	55.1	1.2	21.4	0.2
2.3-3.2	90	106	0.6	59.8	3.0	20.2	0.3
2.3-3.2	120	107	9.0	55.4	2.4	23.3	0.6
2.3-3.2	157	85.3	11.9	54.6	0.7	22.1	4.2
2.3-3.2	183	94.6	3.4	52.3	1.3	19.6	0.6
2.3-3.2	210	67.9	11.4	53.6	0.5	18.8	1.4
Formation							
water	1	nd	na	56.7	na	nd	na
Ctrl 2.3-3.2	45	nd	na	57.9	na	nd	na
Ctrl 2.3-3.2	90	nd	na	62.5	na	nd	na
Ctrl 2.3-3.2	120	nd	na	57.4	na	nd	na
Ctrl 2.3-3.2	157	nd	na	61.0	na	nd	na
Ctrl 2.3-3.2	183	nd	na	55.2	na	nd	na
Ctrl 2.3-3.2	210	nd	na	58.6	58.6	nd	na

Table 3. Continued

Sample ID*	Time	TDC		DIC		DOC		pH		DO	
meters	days	Ave. mg/L	S.D.	mg/L	S.D.	mg/L	S.D.	mg/L	S.D.	mg/L	S.D.
Amended											
water	1	72.9	0.1	69.6	0.4	3.36	0.5	nr	na	nr	na
2.3-3.2	45	72.1	0.9	68.1	0.4	4.02	0.6	7.18	0.0	4.0	0.2
2.3-3.2	90	70.9	0.2	66.7	0.7	4.51	0.7	7.58	0.0	2.2	0.8
2.3-3.2	120	77.8	8.9	72.4	11.4	5.34	2.5	7.29	0.1	1.90	1.8
2.3-3.2	157	72.0	4.7	66.5	6.4	5.43	2.4	7.19	0.1	0.07	0.0
2.3-3.2	183	66.9	2.7	63.3	0.9	3.6	2.6	7.23	0.1	0.86	1.4
2.3-3.2	210	84.7	7.7	82.9	8.3	1.77	1.0	7.11	0.1	0.07	0.0
Formation											
water	1	74.0	na	71.1	na	3.01	na	nr	na	nr	na
Ctrl 2.3-3.2	45	74.9	na	71.6	na	3.27	na	7.24	na	3.44	na
Ctrl 2.3-3.2	90	73.8	na	70.3	na	3.54	na	7.63	na	4.24	na
Ctrl 2.3-3.2	120	74.1	na	71.0	na	3.10	na	7.49	na	4.54	na
Ctrl 2.3-3.2	157	66.7	na	64.7	na	2.02	na	7.45	na	5.09	na
Ctrl 2.3-3.2	183	66.4	na	61.8	na	4.64	na	7.48	na	3.34	na
Ctrl 2.3-3.2	210	65.4	na	61.4	na	4.05	na	7.54	na	6.35	na

Table 3. Continued

Sample ID*	Time	SOC**		SIC		S^{-}	
meters	days	Ave. wt%	S.D	Ave. wt%	S.D	Ave. wt%	S.D.
2.3-3.2	1	0.19	0.01	nr	na	0.01	0.01
2.3-3.2	45	0.13	0.02	nr	na	0.01	0.01
2.3-3.2	90	0.17	0.07	nr	na	0.02	0.01
2.3-3.2	120	0.17	0.01	nr	na	nd	na
2.3-3.2	157	0.16	0.02	14.8	0.53	0.02	0.01
2.3-3.2	183	0.16	0.00	14.2	0.38	0.01	0.01
2.3-3.2	210	0.20	0.03	13.5	1.5	0.01	0.02
Ctrl 2.3-3.2	210	0.17	na	14.8	na	0.01	na

* Sample ID indicates the depth interval sediments were collected.

** Weight % adjusted to account for the weight loss that occurred due to leaching of carbonate minerals.

nr indicates data not recorded

nd indicates not detectable

na indicates not applicable

Table 3. Continued

Sample ID*	Time	NO_3^--N		$SO4^{2-}$		Br^-	
meters	days	Ave. mg/L	S.D.	Ave. mg/L	S.D.	Ave. mg/L	S.D.
Amended							
water	1	104	0.0	55.1	0.0	21.9	0.0
4.9-5.8	45	101	2.4	89.0	6.8	21.5	0.6
4.9-5.8	90	102	3.7	93.0	8.2	21.0	0.8
4.9-5.8	120	93.7	13.7	93.3	14.6	23.3	1.9
4.9-5.8	157	71.3	20.5	93.1	8.5	22.0	1.2
4.9-5.8	183	56.2	25.5	94.7	15.9	21.9	2.7
4.9-5.8	210	50.9	32.3	88.7	2.7	20.2	0.9
Formation							
water	1	nd	na	56.7	na	nd	na
Ctrl 4.9-5.8	45	nd	na	75.3	na	nd	na
Ctrl 4.9-5.8	90	nd	na	88.6	na	nd	na
Ctrl 4.9-5.8	120	nd	na	95.0	na	nd	na
Ctrl 4.9-5.8	157	nd	na	105	na	nd	na
Ctrl 4.9-5.8	183	nd	na	102	na	nd	na
Ctrl 4.9-5.8	210	nd	na	104	104	nd	na

Table 3. Continued

Sample ID*	Time	TDC		DIC		DOC		pH		DO	
meters	days	Ave. mg/L	S.D.	Ave. mg/L	S.D.	Ave. mg/L	S.D.	Ave. mg/L	S.D.	Ave. mg/L	S.D.
Amended											
water	1	72.9	0.07	69.6	0.35	3.36	0.46	nr	na	nr	na
4.9-5.8	45	71.9	1.95	68.8	1.65	3.20	0.31	7.14	0.02	0.2	0.1
4.9-5.8	90	73.3	3.08	67.6	4.67	5.75	1.68	7.58	0.08	0.1	0.1
4.9-5.8	120	80.4	13.2	77.2	14.9	3.17	1.63	7.38	0.10	0.06	0.03
4.9-5.8	157	79.9	8.52	77.2	6.41	2.79	3.27	7.30	0.08	0.08	0.04
4.9-5.8	183	77.5	15.7	79.3	16.0	3.6	na	7.30	0.09	0.06	0.04
4.9-5.8	210	98.3	18.9	94.2	18.9	3.94	1.42	7.34	0.10	0.05	0.02
Formation											
water	1	74.0	na	71.1	na	3.01	na	nr	na	nr	na
Ctrl 4.9-5.8	45	75.8	na	71.2	na	4.58	na	7.04	na	0.02	na
Ctrl 4.9-5.8	90	73.3	na	65.1	na	8.23	na	7.54	na	0.11	na
Ctrl 4.9-5.8	120	70.5	na	67.1	na	3.45	na	7.33	na	2.46	na
Ctrl 4.9-5.8	157	56.9	na	55.7	na	1.19	na	7.92	na	3.44	na
Ctrl 4.9-5.8	183	59.1	na	55.0	na	4.15	na	7.61	na	4.00	na
Ctrl 4.9-5.8	210	56.5	na	52.7	na	3.84	na	7.62	na	2.80	na

Table 3. Continued

Sample ID*	Time	SOC**		SIC		S^-	
meters	days	Ave. wt%	S.D	Ave. wt%	S.D	Ave. wt%	S.D.
4.9-5.8	1	0.43	0.10	nr	na	0.41	0.02
4.9-5.8	45	0.37	0.09	nr	na	0.39	0.01
4.9-5.8	90	0.45	0.03	nr	na	0.42	0.02
4.9-5.8	120	0.45	0.07	nr	na	0.37	0.02
4.9-5.8	157	0.44	0.01	14.0	0.6	0.41	0.03
4.9-5.8	183	0.41	0.03	13.0	0.5	0.36	0.01
4.9-5.8	210	0.48	0.03	11.7	0.5	0.40	0.03
Ctrl 4.9-5.8	210	0.45	na	13.8	na	0.28	na

* Sample ID indicates the depth interval sediments were collected.

** Weight % adjusted to account for the weight loss that occurred due to leaching of carbonate minerals.

nr indicates data not recorded

nd indicates not detectable

na indicates not applicable

Table 3. Continued

Sample ID*	Time	NO_3^--N		SO_4^{2-}		Br^-	
meters	days	Ave. mg/L	S.D.	Ave. mg/L	S.D.	Ave. mg/L	S.D.
Amended							
water	1	104	0.0	55.1	0.0	21.9	0.0
6.7-7.9	45	97.8	1.7	69.4	10.3	21.5	0.9
6.7-7.9	90	99.0	4.3	92.6	5.5	21.2	1.4
6.7-7.9	120	95.1	7.0	93.9	8.9	23.2	1.7
6.7-7.9	157	61.5	11.1	96.1	7.6	22.0	1.7
6.7-7.9	183	66.5	13.6	96.7	17.9	21.9	0.6
6.7-7.9	210	61.1	17.0	120.00	19.47	20.0	1.1
Formation							
water	1	nd	na	56.7	na	nd	na
Ctrl 6.7-7.9	45	nd	na	77.6	na	nd	na
Ctrl 6.7-7.9	90	nd	na	107	na	nd	na
Ctrl 6.7-7.9	120	nd	na	123	na	nd	na
Ctrl 6.7-7.9	157	nd	na	139	na	nd	na
Ctrl 6.7-7.9	183	nd	na	159	na	nd	na

Table 3. Continued

Sample ID*	Tim	TDC		DIC		DOC		pH		DO	
meters	days	Ave. mg/L	S.D.	Ave. mg/L	S.D.	Ave. mg/L	S.D.	Ave. mg/L	S.D.	Ave. mg/L	S.D.
Amended											
water	1	72.9	0.07	69.6	0.35	3.36	0.46	nr	na	nr	na
6.7-7.9	45	70.5	1.57	66.6	1.62	3.20	0.31	7.21	0.05	0.1	0.0
6.7-7.9	90	70.4	2.59	63.5	4.91	6.85	2.83	7.56	0.04	0.0	0.0
6.7-7.9	120	79.4	5.05	74.5	4.11	4.97	1.01	7.37	0.07	0.04	0.01
6.7-7.9	157	84.2	1.74	82.3	2.05	1.87	0.73	7.37	0.01	0.08	0.01
6.7-7.9	183	82.9	8.18	75.2	10.1	7.7	3.2	7.35	0.04	0.06	0.02
6.7-7.9	210	77.9	9.8	72.5	9.9	5.33	0.23	7.35	0.06	0.06	0.02
Formation											
water	1	74.0	na	71.1	na	3.01	na	nr	na	nr	na
Ctrl 6.7-7.9	45	74.8	na	71.0	na	3.82	na	7.13	na	0.05	na
Ctrl 6.7-7.9	90	69.1	na	63.7	na	5.44	na	7.38	na	0.19	na
Ctrl 6.7-7.9	120	66.6	na	62.9	na	3.74	na	7.24	na	1.31	na
Ctrl 6.7-7.9	157	43.5	na	42.1	na	1.40	na	7.86	na	2.53	na
Ctrl 6.7-7.9	183	43.4	na	39.9	na	3.57	na	7.46	na	3.62	na

Table 3. Continued

Sample ID*	Time	SOC**		SIC		S^-	
meters	days	Ave. wt%	S.D	Ave. wt%	S.D	Ave. wt%	S.D.
6.7-7.9	1	0.42	0.02	nr	na	0.49	0.01
6.7-7.9	45	0.42	0.03	nr	na	0.55	0.03
6.7-7.9	90	0.43	0.06	nr	na	0.53	0.02
6.7-7.9	120	0.42	0.03	nr	na	0.53	0.01
6.7-7.9	157	0.42	0.02	14.3	0.5	0.55	0.01
6.7-7.9	183	0.42	0.08	13.9	0.4	0.48	0.02
6.7-7.9	210	0.43	0.03	12.7	0.9	0.53	0.02
Ctrl 6.7-7.9	210	0.40	na	14.5	na	0.40	na

* Sample ID indicates the depth interval sediments were collected.

** Weight % adjusted to account for the weight loss that occurred due to leaching of carbonate minerals.

nr indicates data not recorded

nd indicates not detectable

na indicates not applicable

APPENDIX D

METHOD OF ANALYSIS TO DETERMINE

THE QUANTITY OF SULFIDES

Canfield et al. (1986) developed the Chromium Reduction Method for determination of sulfides. This method was used in the laboratory study to quantify the sulfides present in the EVA sediments. This method entails combining concentrated hydrochloric acid (HCl) and reduced chromium chloride with a measured amount of sediment. Upon the addition of heat, the chromium and acid oxidizes the sulfides present in the sample forming hydrogen sulfide gas (H_2S). The H_2S flows to a bubbling flask containing a zinc acetate and sodium hydroxide solution. The H_2S precipitates out as ZnS. The amount of ZnS precipitated can be accurately measured by titration with an iodine solution (I_2). For these analyses, the concentration of the I_2 solution was 0.1 Normal.

Because the chromium and acid react to liberate sulfides, it is a good way to analyze for sulfide-sulfur (S^--S) and exclude any potential sulfate-sulfur (SO_4^{-2}-S). SO_4^{-2}-S is already in the oxidized state and does not react with the chromium or the acid. Even if sulfate-bearing minerals were present in the sample and dissolved in solution, they would not be converted to a gaseous phase and trapped in the bubbling flask.

The following equations are used to explain the reactions occurring in the analysis and for calculating how much sulfide is present in a sample. Each step in the procedure is outlined in the paper, except for the following reactions and equations.

At the end of the analysis, after the sulfide has precipitated as ZnS, it is dissolved using 30 mL of 6 M HCl. The equation for this reaction is

$$ZnS + 2HCl \rightarrow ZnCl_2 + H_2S \qquad (6)$$

The dissolved H_2S remains in the solution and reacts with I_2 (the titrant). The equation for the reaction occurring during titration is

$$H_2S + I_2 \rightarrow S^0 + 2I^- + 2H^+ \quad (7)$$

This reaction occurs in an acidic solution (Laitinen, 1960).

Using 0.1 N I_2 in this reaction, a calculation can be done to determine how much I_2 reacts with H_2S to make S^0. The following conversions are made to determine the sulfide consumption of a 0.1 N I_2 solution.

0.1 N I_2 = 0.1 equivalents/L I_2 x ½ mole I_2/equivalent x 1 L/1000ml = $5x10^{-5}$ mole I_2/mL

$5x10^{-5}$ mole I_2/mL x 1 mL I_2 = $5x10^{-5}$ mole I_2 (8)

In equation (6) the molar ratio of H_2S to I_2 is one to one.

1 mL 0.1 N I_2 is equivalent to $5x10^{-5}$ mole I_2 or $5x10^{-5}$ mole S^- or 2.5 $x10^{-5}$ mole FeS_2.

The following equation is used to convert this to a usable relationship.

$5x10^{-5}$ mole S^- x 32.06 g/mole x 1000 mg/g = 1.603 mg S^-/mL 0.1 N I_2 consumed (9)

Equation (9) is the relationship used to calculate the quantity of sulfide present. Equation (9) can be taken one step further to calculate the amount of sulfide directly as pyrite (FeS_2), as shown in Equation (10).

2.5×10^{-5} mole FeS_2 x 119.967 mole/g x 1000 mg/g = 2.999 mg FeS_2/ mL 0.1 N I_2 consumed. (10)

The amount of sulfide present in the sample in weight % is calculated by using the constant determined by Equations (9) and (10), the sample weight, and the amount of I_2 consumed.

Wt% S^- = [{mL I_2 consumed* 1.603 mg S^-/mL I_2}/sample weight]*100% (11)

The amount of pyrite in weight percent can be calculated the same way, except using the constant that was determined for pyrite.

Wt% FeS_2 = [{mL I_2 consumed* 2.999 mg FeS_2/mL I_2}/sample weight]*100% (12)

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